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THE EDITOR'S VIEWPOINT

Williams, Forbes, Blau, and Eickenberg (p. 563) found enterococci, chiefly *Str. faecalis,* in the salivas of 21.8 per cent of 206 individuals. Enterococci were also found in 14.2 per cent of positive root canal cultures. The significance of the mouth as a reservoir for enterococci in food handlers and others is discussed. While investigating the enterococci, the authors considered their correlation with salivary lactobacilli and yeast. These small blocks of information, sought for various reasons, are constantly adding to our knowledge of the oral flora.

Hurst (p. 571) found changes occurring in the morphology of oral actinomycetes during observation under the microscope. The morphologic instability is well documented in her excellent photomicrographs. This annoying pleomorphism is one of the factors which makes a study of the dentobacterial plaque and of the oral bioto so difficult. It also points to the inadequacy of morphologic studies of the oral flora.

As the search continues for a substance that will effectively eliminate acidogenic bacteria from the mouth or interfere with their degradation of carbohydrates, more and more investigations indicate that many materials will inhibit acid production in vitro. Forbes, Cox, and Smith (p. 583) add hendecynoic acid to the growing group of suggestions. It is hoped that clinical investigations can be undertaken to evaluate, in vivo, those substances which show effective levels of antibacterial or antienzyme activity in vitro. Unfortunately, clinical studies are expensive and time consuming. In addition, when a group of children has been subjected to substance A they are poor experimental material for test of substance B.

A number of different acids are produced from glucose by oral microorganisms. The possibility of production of citric acid has been considered. Neuwirth (p. 589), however, found that citrates were utilized in incubated saliva-glucose mixtures rather than citric acid being produced by oral bacteria.

Calandra and Adams (p. 593) give the effectiveness of 14 amino derivatives of 2, 3-dichloro-1,4-naphthoquinone in inhibiting acid formation in vitro. The sulfonamides gave the best results at low concentrations. These results again emphasize the great number of possible anticaries agents and the need for in vivo evaluation studies.

Smith, Gardner, and Hodge (p. 596) found the blood fluoride level in Newburgh (1.0 to 1.2 ppm F in water supply) 3 times that of Rochester (0.06 ppm F in water supply) but the urine fluoride level in Newburgh 19 times that in Rochester. Apparently the normal individual can eliminate adequate fluorine through the urine to prevent accumulation to toxic levels in the blood. These are among the studies which are being used to evaluate the artificial fluoridization of water supplies beyond the simple consideration of the immediate effect on caries activity. To date, no valid adverse findings appear to have been reported.

Henry and Peyton (p. 601) report preliminary results in the study of vibration characteristics of the rotating dental instruments. While these studies were conducted under laboratory conditions and not with teeth in their supporting tissues, certain facts become evident. It appears that while decreased pressure with the bur may reduce injurious heat production, it may also increase undesirable vibration. Influence of bur size is suggested and the absence of significant vibration with gas-propelled abrasive (Airbrasive) is noted.

Steck (p. 616) developed a method for measuring changes in vertical dimensions of processed dentures. She applied her method to determine the effects of curing, the shape of teeth, the shape of the denture, methods of packing, and other factors on the vertical dimensions.

Jansen and Visser (p. 622) have investigated the permeability of enamel to a fluorescing dye. They found that the dye per-

meated prism sheaths, lamellae, and tufts in intact enamel of dogs in vivo. They, very wisely, point out that their most careful study does not find evidence of vitality of enamel nor how the dyestuff flows. The well-documented review of past studies of enamel permeability is invaluable to students of this controversial subject.

Jansen (p. 633) describes a better method for preparing very thin ground sections of enamel. This technic may permit more complete observation of calcified enamel structures.

Wentz, Weinmann, and Schour (p. 637) studied the epithelial remnants in the molar teeth of the rat. They classified the remnants found in 50 per cent of the specimens into three types: small resting, proliferating, and differentiating. Fewer remnants were found in older rats, but the differentiating type increased with age. The authors discuss Hertwig's sheath as a source of rests but also point out that the gingival epithelium or the epithelial attachment may contribute epithelial islands.

The Proceedings of the INTERNATIONAL ASSOCIATION OF DENTAL RESEARCH are published in this issue (p. 649). The President's address sketches the growth of science in general and dental science in particular. One hundred twenty-three abstracts should give the reader a good view of dental science in progress.

H. B. G. R.

A STUDY OF THE SIMULTANEOUS OCCURRENCE OF ENTEROCOCCI, LACTOBACILLI, AND YEASTS IN SALIVA FROM HUMAN BEINGS

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THE MICROORGANISMS that may occur in the mouth have been studied almost continuously in a variety of ways and for a number of reasons ever since the original observations by Leeuwenhoek in 1683. Most of the investigations were undertaken in order to (1) obtain information on the etiologic agents of specific infections in the mouth or oral manifestations of infections in some other part of the body, and (2) determine the relative occurrence of some organism hitherto not described as being found in the mouth. While these studies have contributed toward a better understanding of the type of microbial flora that may exist in the oral cavity, few attempts have been made to determine whether there are ecologic relationships between the organisms of particular interest and other forms in the same flora. The recent report of Hemmens, Blayney, Bradel, and Harrison,[5] on the microbic flora of the dental plaque is a good example of such a study.

The endodontia clinic of this school requested identification of cultures of microorganisms obtained from several cases which did not respond to treatment with antibiotics. The cultures were obtained from the root canals of teeth being treated by topical application (within the root canal) of a combination of penicillin and streptomycin in high concentration.[2] Cultural and biochemic study indicated that the organisms belonged to the enterococcus group of streptococci, the principal species being *Streptococcus faecalis*. Since the saliva was the probable source of the organisms, a study was made to determine how frequently enterococci could be recovered from the salivas of apparently healthy persons. Inasmuch as facilities were available for making lactobacillus counts on pH 5.0 tomato juice agar, a quantitative determination of lactobacilli and yeasts was made simultaneously on the same saliva samples.

METHODS

Enterococci.—The procedure for isolation and identification of enterococci is given in outline form in Fig. 1. Saliva was collected on arising in the morning and sent to the laboratory where each sample was thoroughly mixed by means of a shaking machine for 15 minutes and then by a pipette, just prior to sampling for culture. A tube of enterococci-presumptive broth[16] (Difco) was inoculated with 0.1 c.c. of the mixed saliva. The tubes were incubated at 45° C. for 24 to 48 hours, growth and change in indicator being positive presumptive

This investigation was conducted in part under a contract between the Office of Naval Research and the Thomas W. Evans Museum and Dental Institute School of Dentistry of the University of Pennsylvania.

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evidence for enterococci. Negative tubes were discarded. An inoculum of 0.1 c.c. from all positive tubes was transferred to 0.1 per cent methylene blue milk (MBM) and the latter incubated at 37° C. for 48 hours. The presence of gram-positive streptococci and reduction of methylene blue was taken as confirmatory evidence of enterococci. Identification was initiated by transferring 0.1 c.c. from the MBM tube to a stroke-pour blood agar plate.[10] After incubation for 48 hours, a single colony, or colonies if no changes in blood occurred, was transferred to 0.2 per cent glucose broth (pH 7.2) and incubated at 45° C. for 24 hours. This culture was used to inoculate a series of media which served to confirm further the presence of enterococci and to assist in differentiation of species.[9, 11] Serologic identification of the cultures was not undertaken. It is believed that the biochemic and other cultural tests conducted were sufficient to identify the isolated streptococci as enterococci.

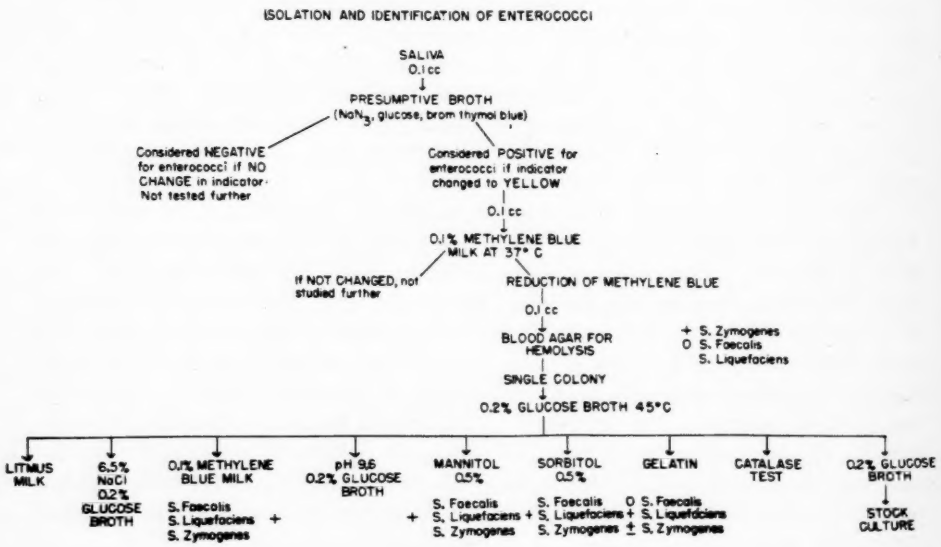

Fig. 1.

Lactobacilli and Yeasts.—Saliva samples were diluted 1:5 and 1:50 in dextrose broth pH 5.0, and then 0.1 c.c. of each dilution was spread on tomato juice agar (pH 5.0) plates[3] and incubated aerobically at 37° C. for 4 days. Lactobacillus counts were based on colony recognition and smears, cultures being made if doubt existed. If a plate contained only 1 or 2 predominant colony types, sufficient growth was removed from each of the colony types to prepare a suspension of the organisms in sterile 0.85 per cent NaCl solution. This suspension was tested serologically, by means of the spot agglutination technic, for the presence of lactobacillus antigenic components A,B,C,D,E,F,G,H, and I.[8, 14, 15] If spontaneous agglutination was noted, organisms from the suspension were inoculated into tomato juice broth and prepared for the agglutination tests by

washing and filtering. Yeast counts were made on recognition of colonies which showed gram-positive budding cells. No attempt was made to identify the species of the lactobacilli or yeasts.

RESULTS

The methods used for isolation and confirmation of enterococci in saliva differ from those used by investigators[4, 16] who studied the occurrence of enterococci in polluted waters or in other sources. A preliminary trial of the confirmatory medium of Winter and Sandholzer[16] yielded 35 false positives out of 48 positive tubes, all being inoculated from positive presumptive broth tubes. Tests with other media than this confirmatory medium culminated in acceptance of presumptive broth and methylene blue milk employed in consecutive steps. The validity of this procedure was checked by the series of media, which further revealed characteristics and aided in identification. The results in the present study show a reduction in false positives to 10 out of 76 (11.8 per cent). Tests of the sensitivity of the method were made by inoculating a strain of *Str. faecalis* into a sample of saliva found to be free of enterococci. The data indicate that it is possible to demonstrate the presence of this strain if there were as few as 250 viable cells in each c.c. of saliva. This saliva was not found to have an inhibitory effect on the strain employed.

Saliva samples obtained from 206 persons over a period of 3 months were studied for the presence or absence of enterococci, lactobacilli, and yeasts. The findings concerning enterococci are presented in Table I. The group was arbitrarily divided into 3 age categories, 4-13 (Column *A*), 14-20 (Column *B*), and 21 years or over (Column *C*); these sub-groups contained 98, 58, and 50 persons, respectively. Salivas from 45 out of 206 persons (21.8 per cent) were positive for enterococci at least once. The occurrence of enterococci in the salivas of the Groups *B* and *C* was slightly higher than in Group *A*. The ages, number of samples, and number of times each sample was positive are noted opposite the species of enterococci found in the salivas. Saliva samples were positive more frequently in *B* than in the other 2 groups. The salivas from 5 persons in Group *B* were positive every time they were studied. Note particularly the one person aged 19 whose saliva was positive each time it was tested. *Str. faecalis* was identified in 82.2 per cent of the positive salivas, *Str. liquefaciens* in 11.1 per cent, and *Str. zymogenes* in 6.6 per cent. *Str. durans* was not found even though all negative (no reduction) MBM tubes were subcultured on stroke-pour blood agar plates.

The number of salivas, taken at different time intervals, was 199, 132, and 102, respectively, for Groups *A*, *B*, and *C*, making a total of 433 samples. The number of positive saliva samples was 66, or 15.2 per cent. Since there was more than one saliva sample from the majority of persons this figure cannot be compared with the number of persons recorded as positive (21.8 per cent). Note that there were more positive saliva samples from Group *B* than from other groups.

The data from the estimations of the numbers of lactobacilli and yeasts in the salivas were analyzed statistically to determine whether there was a

relationship with the presence or absence of enterococci in the samples. The analyses were restricted to results from those persons who had supplied more than one saliva sample and whose samples were found to contain any of the species of enterococci at least once. Consideration was given first to possible relationships between the groups and secondly to all persons, regardless of age, whose saliva samples were found to contain only *Str. faecalis* of the enterococcus group.

TABLE I

FREQUENCY OF OCCURRENCE OF ENTEROCOCCI IN SALIVAS FROM INDIVIDUALS OF DIFFERENT AGES

	AGE GROUPS									TOTALS AND PERCENTAGE
	A 4-13 years			B 14-20 years			C 21 years and over			
PERSONS TESTED	98			58			50			206
PERSONS POSITIVE	21 (21.4%)			13 (22.4%)			11 (22.2%)			45 (21.8%)
	AGE	NO. OF SALIVAS	TIMES POSITIVE	AGE	SALIVAS	POSITIVE	AGE	SALIVAS	POSITIVE	
STREPTOCOCCUS FAECALIS	4	3	2	14	2	1	21	3	2	37/45 82.2%
	6	5	1	14	3	3	25	2	1	
	6½	2	1	15	3	1	26	1	1	
	8	3	1	15	3	3	28	3	1	
	9	1	1	15	3	1	29	2	2	
	10½	2	1	16	4	1	30	3	1	
	11	5	1	16	3	3	30	3	1	
	11	1	1	18	3	1	30	2	2	
	12	1	1	18	1	1	33	1	1	
	12	2	1	19	7	7	34	1	1	
	12	3	2	20	3	2				
	13	5	1							
	13	4	1							
	13	2	1							
	13	1	1							
	13	1	1							
STREPTOCOCCUS LIQUEFACIENS	6	1	1	15	3	1	23	2	1	5/45 11.1%
	13	1	1							
	13	3	2							
STREPTOCOCCUS ZYMOGENES	7	2	2	20	2	2				3/45 6.6%
	13	3	1							
NUMBER SALIVAS TESTED	199 (from 1-5 samples)			132 (from 1-7 samples)			102 (from 1-6 samples)			433
NUMBER SALIVAS POSITIVE	25 (12.6%)			27 (20.4%)			14 (13.7%)			66 15.2%

Examination of the data from Groups *A*, *B*, and *C* revealed that, after application of the above criteria, there were too few counts in Group *C* to apply statistics, and yeast counts in each of these groups were too infrequent to be analyzed. A summary of the data used for analysis of the lactobacillus counts for Groups *A* and *B* is presented in Table II. It was found that counts from 14 out of 21 persons in *A* and 10 out of 13 persons in *B* could be used. Enterococci had been found in 18 saliva samples, 25 samples being negative in Group *A*, while in Group *B* there were 15 positive and 14 negative samples. Lactobacilli were found in all samples, but there were differences in mean counts. Statistical analyses indicated that the difference between the means in Group *A* is not significant, whereas the almost threefold difference between the means in Group *B* is significant at the 0.05 level.

Data from persons whose salivas were found to contain *Str. faecalis* on one or more occasions were used to construct Table III. There were 21 persons in the group, from whom 58 saliva samples were analyzed. These were subdivided into those samples which were positive or negative for the enterococcus. The presence or absence of lactobacilli and yeasts for each of the samples, the range of the counts, and the means are given. Lactobacilli were found 22 times out of 23 saliva samples, all of which were positive for enterococci, the mean count being 332,000 per c.c. Lactobacilli were in all of the samples not found to contain enterococci, the mean count being 150,000. The difference between these means was found to be significant at the 0.05 level.

TABLE II

RELATIONSHIP BETWEEN OCCURRENCE OF ENTEROCOCCI AND NUMBERS OF LACTOBACILLI

GROUP	NO. OF COUNTS	NO. TIMES ENTEROCOCCI FOUND	LACTOBACILLI	
			RANGE x 10^3	MEAN x 10^3
A (4-13 years of age)	18	Str. faec. - 15 Str. liq. - 3 Str. zym. - 1	2 - 1,800	278
	25	None	0.05 - 850	150
B (14-20 years of age)	15	Str. faec. -13 Str. liq. - 1 Str. zym. - 1	0.05-1,978	341
	14	None	0.05-595	133

The differences between means found for the group in Table III and Group *B* (Table II) seem to indicate that if enterococci are present there is likely to be a higher lactobacillus count than if enterococci are absent. It was found also that there was a significant difference between the total lactobacillus counts for Groups *A*, *B*, and *C*. Group *B* was found to have the greatest mean numbers of lactobacilli, *A* the next, and *C* the least.

TABLE III

RELATIONSHIP BETWEEN OCCURRENCE OF STR. FAECALIS AND NUMBERS OF LACTOBACILLI AND YEASTS IN 58 SALIVA SAMPLES FROM 21 PATIENTS

STR. FAECALIS	LACTOBACILLI			YEASTS		
	NO. SAMPLES PRESENT	RANGE x 10^3	MEAN x 10^3	NO. SAMPLES PRESENT	RANGE x 10^3	MEAN x 10^3
FOUND IN 23 SAMPLES	22	0.05 - 1978.0	332.0	9	0.1 - 84.0	8.2
NOT FOUND IN 35 SAMPLES	35	0.05 - 10000	150.0	17	0.1 - 250.0	24.8

Yeasts were present in only 9 of the 23 samples (Table III) positive for the enterococcus, the mean being 8,200 per c.c., but yeasts were found in 17 out of the 35 samples which were found to be negative for the enterococcus, the mean being 24,800. The difference between these means was found to be significant at the 0.05 level. Thus, it would appear that when enterococci are present there are likely to be lower numbers of yeasts.

The spot agglutination tests with suspensions of lactobacilli, prepared either from a single colony on the tomato juice agar plate or from the broth culture, indicated that many of the strains tested contained antigenic com-

ponents similar to those found previously. A total of 40 colonies were studied, and of these, 20 contained one or more of the antigenic components A,B,C,F,G, and H. It was noted that 12 of the strains contained the F component and 7 contained the H component. None contained the D,E, or I component.

DISCUSSION

Wohlfiel[17, 18] recovered enterococci from the surfaces of the gingivae in 24.2 per cent and from the surfaces of the tonsillar and pharyngeal areas in 18.5 per cent of the persons he examined. While the numbers cultivable from the gingivae compare favorably with our results from saliva, there is some doubt as to whether the strains Wohlfiel identified were really enterococci because of the methods used in identification. As far as we are aware no studies of the occurrence of enterococci in the oral cavity have been made since the distinguishing characteristics of enterococci were described by Sherman.[11, 12]

Enterococci have been reported previously in cultures from infected root canals by Houston,[6] Evans and Chinn,[1] and possibly others, but no special records were kept on the frequency of occurrence. The endodontia clinic determined the presence or absence of enterococci whenever positive root canal cultures were obtained. During the school year of 1948-1949, 14.2 per cent of the positive cultures contained enterococci. It may well be that the use of a mixture of streptomycin and penicillin in treatment of these root canals led to a higher incidence of enterococci than has been noted previously. It is well known that the enterococci are resistant to the effects of penicillin and streptomycin. However, the finding of enterococci in the salivas of several of the patients whose root canal cultures were positive suggests that the antibiotics inhibited the other microorganisms in the root canals and thus simplified the recovery of enterococci.

Enterococci have been found in a large number of pathologic processes (of microbial origin) in humans and since these organisms are known to be common inhabitants of the intestinal tracts of humans and lower animals it is usually presumed that the pathogenic strains are derived from these sources. While no attempt was made in this study to investigate the infectivity of the species isolated from saliva, it may be assumed that they are as potentially pathogenic as those in the intestinal tract.

The relative frequency of occurrence in saliva indicates that the oral cavity may be considered as an additional reservoir. The organisms may gain access to the tissues by a variety of routes from the oral cavity, leading, possibly, to a simpler explanation of the source of the infecting organisms in reported cases of otitis media, mastoiditis, meningitis, and some cases of endocarditis. In view of this possibility, it would be interesting, when the latter types of infections are found to be caused by enterococci, to determine the frequency of occurrence of these bacteria in the mouth or saliva. The involvement of enterococci in certain outbreaks of food poisoning, and the recent report of Osler, Buchbinder, and Steffen,[7] on experimental production of food poisoning in man, suggests, in view of our findings, that although the hands of food handlers may not be contaminated with enterococci the possibility of the persistence of these

organisms in the saliva of such persons should be considered. Coughing, sneezing, or talking during food preparation by a person carrying the organisms in saliva could contaminate the food.

The analyses indicate that high lactobacillus and low yeast counts are likely to be found in saliva when enterococci are present. Our data, however, do not indicate that enterococci in saliva may serve as an index of the presence or absence of cavities in the teeth of the patients from whom saliva was obtained. On the other hand, Wohlfiel,[17, 18] in discussing dental caries in man, and Wakeman, Smith, Zeeplin, Sarles, and Phillips,[13] in a report on microorganisms in decayed teeth of cotton rats, have indicated that enterococci may have a role in the initiation or extension of decay in teeth. Hemmens, Blayney, Bradel, and Harrison[5] reported a more frequent occurrence of aciduric streptococci than lactobacilli in bacterial plaques from teeth which became carious, but also noted that a similar frequency of streptococci occurred in plaques from areas which did not become carious whereas lactobacilli were much less frequent. It was concluded that the case for streptococci was not as strong as that for lactobacilli. Since enterococci are known to occur in saliva and are aciduric and acidogenic, it would have been interesting if the occurrence of enterococci, among the aciduric streptococci, could have been assayed in these studies of the bacterial plaque. It is realized that an inconstant variation exists in the occurrence of microorganisms in saliva as compared with the bacterial plaque on teeth; therefore, it would appear that further study is desirable.

SUMMARY

Enterococci were cultivable from the salivas of 45 of 206 persons (21.8 per cent). The order of the frequency of occurrence of the various species was *Str. faecalis, Str. liquefaciens,* and *Str. zymogenes.* The salivas from the age group 14 to 20 years were found to contain enterococci slightly more often and more consistently than the age groups 4 to 13 years and 21 years and over.

Isolation of enterococci from infected root canals was possible from 14.2 per cent of positive cultures and in many instances these organisms were cultivable simultaneously from the saliva of the same patient.

The occurrence of enterococci in saliva appears to be sufficiently frequent that the oral cavity should be considered as a possible reservoir of potentially pathogenic strains in addition to the intestinal tracts of humans and animals.

The significance of the mouth as a reservoir, in so far as food handlers are concerned, and the possible participation of enterococci in dental caries are discussed.

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MORPHOLOGIC INSTABILITY OF ACTINOMYCETES ASSOCIATED WITH ENAMEL

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INTRODUCTION

HISTOLOGIC sections of preclinical microscopic carious lesions have shown plaques containing filamentous organisms on the enamel surface and coccoidal forms within the enamel (Frisbie, Nuckolls and Saunders,[1] Nuckolls and Frisbie,[2] Frisbie and Nuckolls.[3]) During bacteriologic studies prompted by the histologic observations, it was found that actinomycetes, morphologically similar to the filamentous forms, could be cultivated both from the surface of such lesions and from sound enamel surfaces. Upon secondary cultivation some strains of these actinomycetes exhibited diphtheroid and coccoidal cells in addition to filamentous cells. In some transfers the coccoidal cells would become predominant while in later transfers the filamentous cells would again appear. These changes could not be correlated with the age of the culture nor the type of media employed. The transition was sometimes accompanied by a change from a rough to a smooth colony type but this was not always the case.

This association suggested that there might be some significant relationship between the filamentous and coccoidal forms characteristic in the histopathology of preclinical caries. Two explanations might be offered for the association observed in cultures.

The first possibility was that these actinomycetes were highly pleomorphic, and that the two forms represented the extremes of pleomorphic variation. If this were true, the coccoidal forms observed histologically within the carious lesion conceivably might be progeny of the filamentous forms seen on the surface. Were this the case, the bacterial flora of preclinical caries might therefore be more simple than heretofore suspected. Pleomorphism is common among some members of the actinomycete group. The tendency of some strains of parasitic actinomycetes, particularly those of the Wolff-Israel type,[4] to assume a rod or diphtheroid morphology during cultivation has often been observed. Moreover, Ørskov,[5] in a detailed study on the morphology of the actinomycete group as a whole, observed that some strains among his Group IIb developed cylindrical, oval, or spherical cells capable of developing into filaments. Coccoidal forms have since been observed among certain nonsporulating strains of actinomycetes by Jensen[6, 7] and Erikson.[8] It, therefore, seemed that strains cultivated from carious surfaces might be capable of a comparable degree of pleomorphism.

The second possibility was that the coccoidal forms were bacteria of a different genus, difficult to separate from the actinomycetes in culture. This might imply a commensal relationship between the two forms observed histologically which conceivably might be of significance in the development of the

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preclinical carious lesion. It has generally been accepted that filamentous bacteria, and particularly oral actinomycetes[9] are difficult to isolate from other bacteria by virtue of their physical form. Gies and collaborators[10] observed that *Cladothrix placoides,* an organism cultivated from teeth which would now probably be classified as an actinomycete, was difficult to isolate from other bacteria. They argued that these organisms contributed to the carious plaque by physically binding other more acidogenic bacteria to the tooth surface. Although there is, at present, little understanding of bacterial associations in disease, better understanding of bacterial metabolism than was available in the time of Gies makes this argument now seem oversimplified.

Associative relationships have been suspected in the disease of actinomycosis. The frequent occurrence of other bacteria, together with actinomycetes in actinomycotic lesions, has led some to believe that bacterial association contributes to the pathogenesis of this disease. Again, difficulty has been encountered in separating the actinomycetes from the accompanying bacteria. Vawter[11] has pointed out that *Corynebacterium pyogenes,* which sometimes occurs with *Actinomyces israeli* in actinomycotic lesions, may persist indefinitely in cultures of *A. israeli* without recognition. It therefore seemed reasonable to believe that coccoidal forms might accompany the actinomycetes cultivated from preclinical carious enamel as generically separate organisms.

To determine whether the coccoidal forms were pleomorphic actinomycetes or whether they were generically separate organisms, direct observations were made on the multiplication of single filamentous cells and of single coccoidal cells.

METHOD OF OBSERVATION

The growth of individual multiplying cells was observed directly under the microscope at time intervals. At each observation a photomicrograph was taken to provide a permanent graphic record of growth, free from the subjectivity of written descriptions and sketches. The observations reported are based on approximately three hundred sequential photomicrographs.

In the initial studies, growth was observed on agar spread on slides, according to the method of Ørskov.[12] The Difco proteose peptone No. 3 agar used was passed through qualitative grade filter paper before sterilization to provide the optical transparency essential to photomicrography. Laked blood was incorporated with the agar in a final concentration of 10 per cent. Inocula were taken either from broth cultures or suspensions made from agar slants. Between observations the slides were kept in Petri dishes to prevent excessive drying of the agar film. Because carbon dioxide seemed to promote more rapid growth of the strains studied, the slides were incubated at 37° C. in a candle jar.

As the work progressed, the Ørskov agar slide technic was found subject to several limitations. The most serious of these was the rapidity with which the agar film dried, therefore terminating growth. Another objection was the unavoidable change from incubator temperature to room temperature each time an observation was made. Although this did not appear to interfere with growth, a possible effect on the growth pattern could not be disregarded.

A microculture chamber designed to overcome these difficulties, as well as to meet the critical optical requirements for photomicrography, was used in the later studies. It its preparation a small drop of the laked blood proteose peptone agar was spread in a thin film on a flamed slide. A drop of malt agar was spread about ½ inch away from the blood agar. The cells to be observed were inoculated on the blood agar, while *Saccharomyces cerevisae* was inoculated on the malt agar to provide an atmosphere of CO_2 within the microchamber comparable to that of the candle jar. Number 1 cover slips were then cleaned, flamed, and, when cool, dropped onto each inoculated agar film. The junction of the two cover slips was sealed with paraffin and the outer margins of the cover slips were sealed to the slide with paraffin. To permit some access to oxygen, a small pinhole was made at one point in the paraffin seal. The space between the cover glass and the slide which separated the two agar films permitted gaseous exchange. This slide was placed in the warm stage thermostatically regulated to 97° to 98° F., and the fields to be studied were noted by means of the Vernier scale on the mechanical stage. From four to eight fields were followed in each series of observations. An attempt was made to distribute the fields chosen throughout the agar film area. Only fields containing organisms not more than about twenty in number, and reasonably well distributed, were used.

A high dry 43 X objective was usually employed with a 15 X wide-field Hyperplane ocular. On several trials an oil immersion objective was used with the microchambers, but it failed to give better resolution and its depth of focus was too limited. The photomicrographs were taken at a final magnification of approximately 800 X on 4″ × 5″ film from which contact prints were made.

Two of the cultures obtained from enamel scrapings were selected for study: *Culture 85* was chosen to represent those in which the cell type was relatively constant and which would provide a pattern with which the more variable cultures could be compared. It was cultivated from scrapings taken from a preclinical carious enamel lesion on the proximal surface of a human molar. The primary colony was rugoid, raised, and white. It was grown on a blood agar plate incubated in a sealed Petri dish can for 10 days at 37° C. In subculture it grew without blood, on almost any nutrient media in 2 or 3 days. The cells in smears were predominantly gram-positive filaments of varying lengths which were frequently branched. They were accompanied by a few shorter almost bacillary cells. *Culture 80* was chosen as representative of those in which the cell types were so highly variable as to suggest the possibility of a mixed culture. It was cultivated from scrapings taken from the molar teeth of a Syrian hamster. The primary colony was white, smooth, glistening, and not readily distinguishable from those of other bacteria frequently found among the oral flora. It was cultivated on a blood agar plate incubated in a candle jar for 10 days at 37° C. Later it was found also to grow on most media aerobically in 2 or 3 days, though carbon dioxide seemed to promote better growth. Smears made from cultures contained (1) filaments of different lengths, sometimes with globular ends, (2) small diphtheroids, (3) coccobacilli, and (4) coccoidal cells varying greatly in size and regularity of outline. All

forms were gram-positive. Sometimes all cell types were found in each colony; sometimes only coccoidal cells were found in all colonies. There was some tendency for the filamentous phase to develop in liquid media and the coccoidal phase to develop on solid media, but this was not constant.

OBSERVATIONS

Culture 85 (Plate I).—Because this culture produced a flocculent growth in broth, the cells observed on the inoculated agar slide were usually in clusters (Plate I, Fig. 1). Only a few single isolated cells could be found and these failed to grow. At the start of the observations the cells were about 5 μ to 10 μ in length and 1 μ in thickness. Different portions of the same cell varied in thickness and in apparent density of protoplasm (Plate I, Fig. 1).

Growth of the cells usually began about 3 hours after they had been seeded on the agar slides. A great difference was noted in the rate at which different cells grew in length. Some cells grew very rapidly at first, but later grew rather slowly. Others began to elongate slowly but later grew so rapidly that they ultimately became the longest filamentous cells. By careful observation with regard to near-by reference points it was observed that growth usually occurred from one end of the cell, the other end remaining fixed. The ability to divide varied considerably with different cells. Some divided into two or more cells, when they had reached a length of about 20 μ. Others continued to elongate to almost 30 μ without division. The method of cell division seemed to be a simple transverse fission which took place suddenly. After division had occurred the position of the new cells formed an obtuse angle. This gave the several cells produced from a filament a characteristic zig-zag arrangement (Plate I, Figs. 3 and 4). Soon after division the newly formed cells began to elongate again into filaments (Plate I, Figs. 4, 5, 6). When branches formed, they began as small buds, usually at the end of a filament, though lateral branches sometimes occurred (Plate I, Figs. 4, 5 and 7, 8). Often they continued to grow until they were as long as the cells from which they had stemmed.

Growth of all cells seemed to be only in the plane of the agar surface. Penetration of the agar was never apparent, nor were aerial mycelia, like those produced by the sporulating actinomycetes, ever observed. Colonies were formed as the cells continued to grow and multiply. After about 30 hours' incubation colonies derived from only a few cells were approximately 90 μ in diameter, and consisted of such a congested mass of cells that further observations of individual cells were impossible.

Culture 80.—Growth of this culture was observed when it was initially in (1) its filamentous phase consisting of short filaments, rods, or diphtheroids, and in (2) its coccoidal phase consisting almost entirely of coccoidal cells or coccobacilli.

Growth of the Filamentous Phase (Plates II and III).—As with Culture 85, the cells of the filamentous phase of Culture 80 were often found in clusters in the inocula. Unlike Culture 85, however, single isolated cells usually grew and were selected for observation.

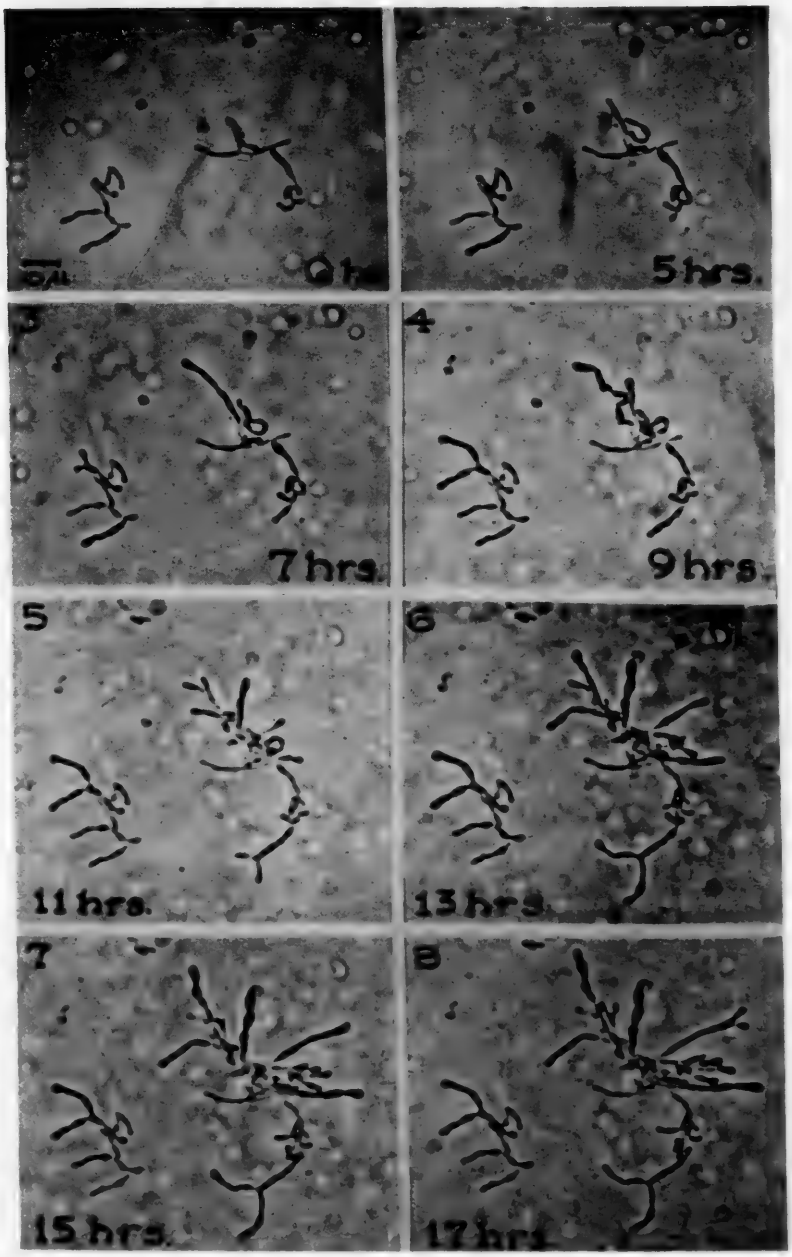

Plate I.—Inoculum: Strain No. 85; 48-hour broth culture. Culture technic: Ørskov agar slide.

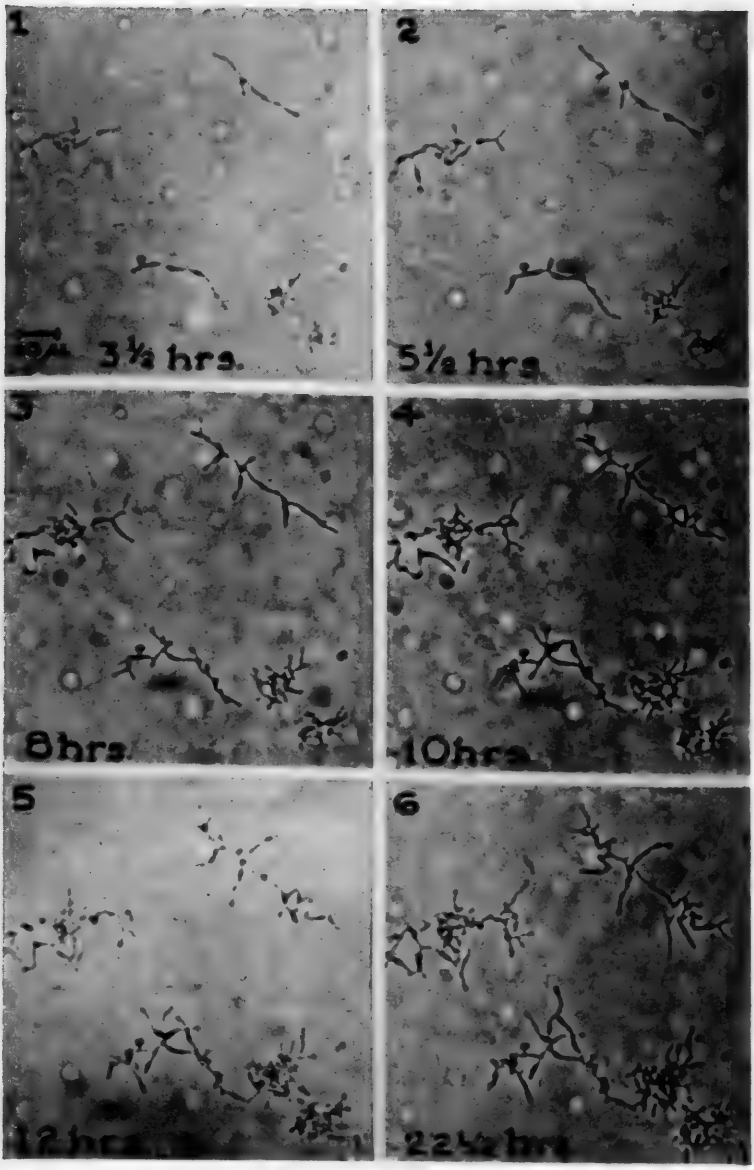

Plate II.—Inoculum: Strain No. 80; 36-hour broth culture, filamentous phase. Culture technic: Ørskov agar slide.

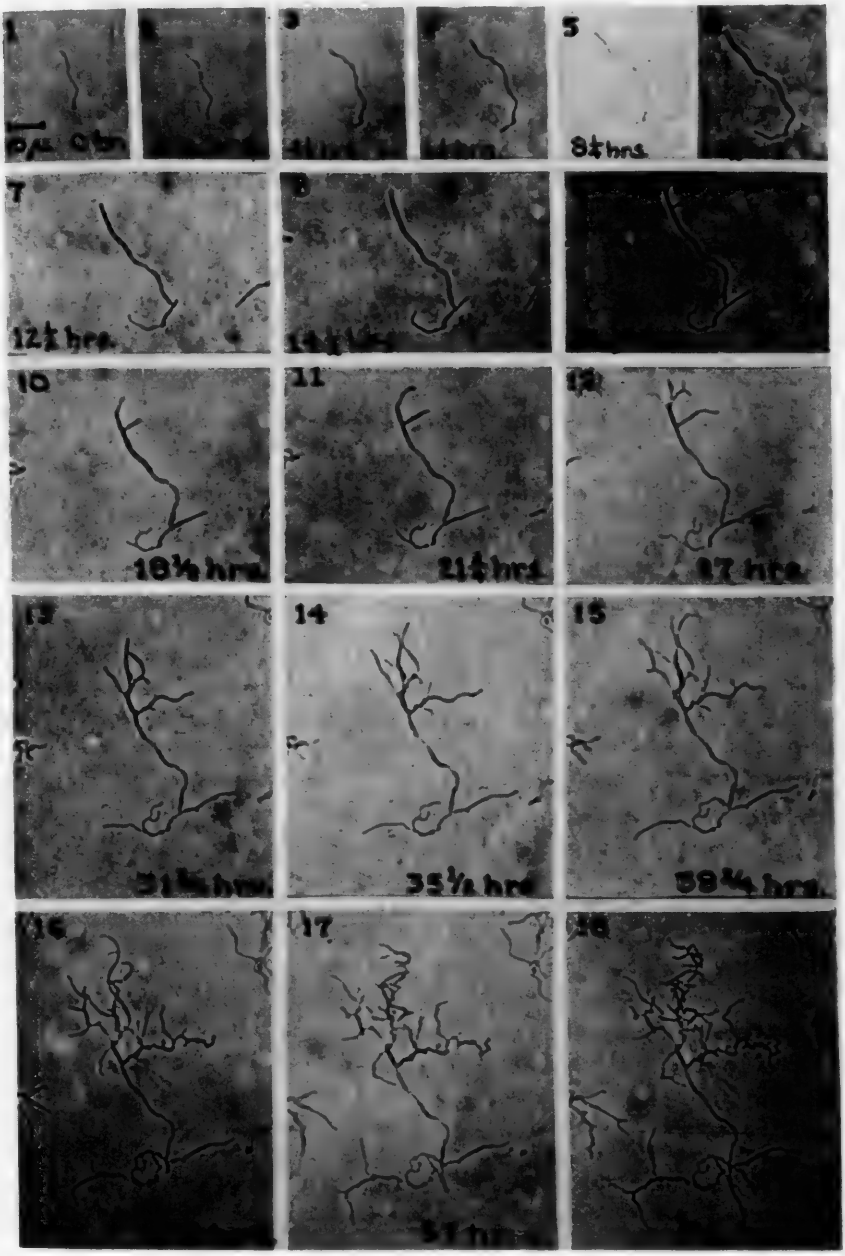

Plate III.—Inoculum: Strain No. 80; 39-hour broth culture filamentous phase. Culture technic: microculture chamber.

At the start of the observations the cells ranged from 5 μ to 20 μ in length. They were somewhat less than 1 μ in width and therefore appeared more delicate than the cells of Culture 85. Like Culture 85 the cells varied somewhat in width and different portions appeared to vary in density (Plate II, Fig. 1). In general, the processes of growth and multiplication were similar to those of Culture 85. Growth usually began about three hours after the start of the observations. Different cells varied considerably in their rate of elongation and in their ability to divide. Cells newly formed by division lay at angles to one another (Plate II, Figs. 2 and 3; Plate III, Figs. 6 and 7). As with Culture 85, the cells grew only in the plane of the agar surface and did not produce mycelia.

In certain minor respects the filamentous phase of Culture 80 differed from Culture 85. Lateral branching occurred more frequently (Plate II, Figs. 2 and 3; Plate III, Fig. 9) while terminal branching seemed to occur with less frequency. The young colonies of Culture 80 consisted of cells less compactly arranged than those of Culture 85. Also they seemed to grow more at random, extending varying lengths in different directions and the cells on the periphery did not show the palisade arrangement observed in young colonies of Culture 85. This difference in colony appearance may have been attributable to certain characteristics in the growth and multiplication of Culture 80's cells: (1) The cells in a colony of Culture 80 varied more in length than did those of Culture 85. Short cells were constantly being produced in the colonies of Culture 80 whereas in Culture 85 short cells were produced less frequently as the young colony proceeded to form. (2) The cells of Culture 80 elongated from both ends rather than from only one. As a result, crossing of growing cells which lay at angles to one another was much more frequent in Culture 80 than in Culture 85.

Coccoidal cells were not observed to be produced from the filamentous phase under the conditions of direct observation. After about 60 hours some of the long filaments had begun to divide into very short cells, (Plate III, Figs. 16 to 18) which did not subdivide further, probably because the over-all rate of growth was beginning to decrease at this time and ceased entirely at the end of 124 hours. It is conceivable that had conditions permitted continued growth, coccoidal cells might have developed.

Growth From the Coccoid Phase (Plate IV).—The coccoidal cells which had developed spontaneously on a blood agar slant were about 1 μ in diameter at the beginning of the observations and were singly dispersed across the agar surface. They began to multiply after the usual lag of about 3 hours (Plate IV, Figs. 1 and 2). Because of their small size, the details of the division process were difficult to observe. There seemed to be two separate processes, either of which could initiate cell division: (1) The coccoidal cell elongated to a coccobacillus which bent in the middle and then divided into two cells lying at an angle (Plate IV, Fig. 2). (2) More frequently, the coccoidal cells seemed to produce one or two small sprouts (Plate IV, Fig. 2). When two sprouts formed, they projected in opposite directions from the cell. Division then occurred, separating the sprout from the coccoidal cell (Plate IV, Figs. 2 and 3). The cells de-

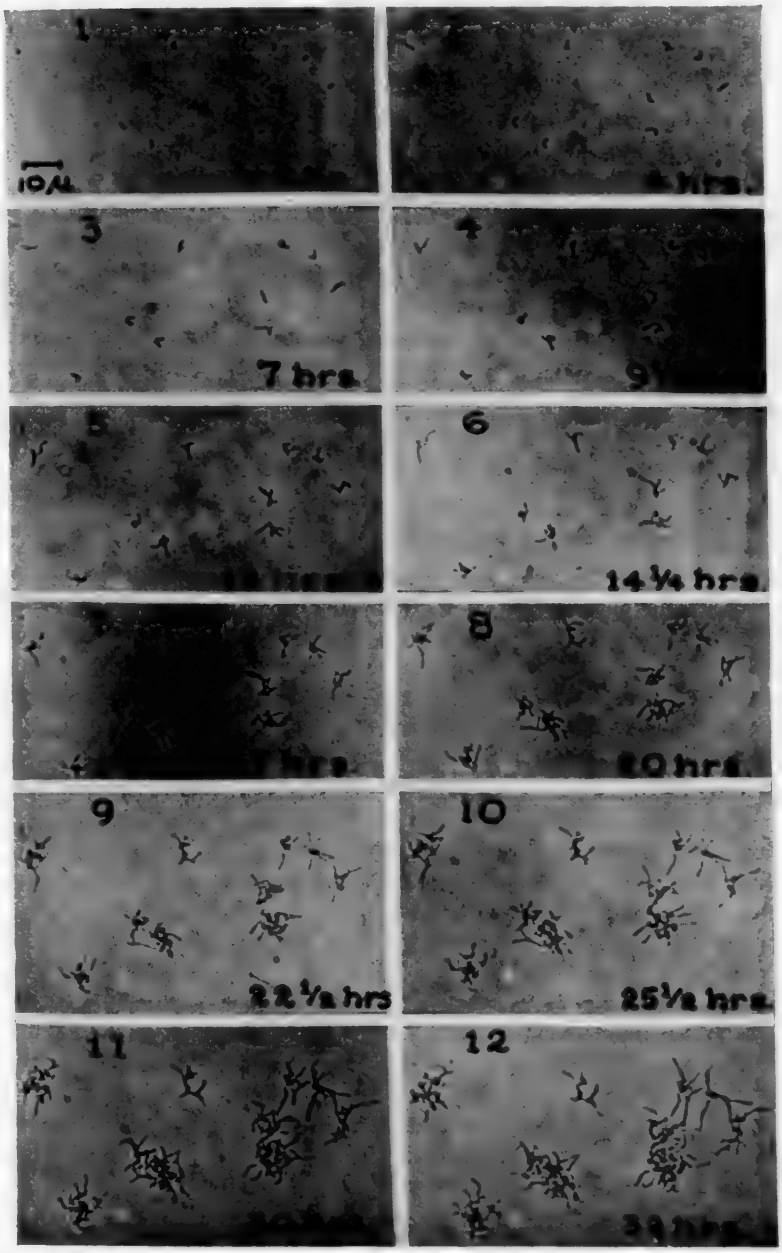

Plate IV.—Inoculum: Strain No. 80; 35-hour blood agar slant culture, coccoidal phase. Culture technic: microculture chamber.

rived from the sprout portions elongated slightly and again divided. As a small colony of cells was built up about the coccoidal mother cell, it became difficult to follow its fate. Sometimes it later appeared to put out another sprout or two but this process was always obscured by the proximity of other multiplying cells in the young colony. Often it was still visible as a small dark body after the colony had reached considerable size (Plate III, Figs. 7 to 12).

The short slender bacillary cells which were derived from either of the two division processes just described were usually about 2 μ in length. As multiplication continued, the cells of each new generation became slightly longer than those of the preceding generation before they divided. Around the twenty-fourth hour of incubation they began to branch. From this time on the growth sequence was identical to that which had been observed in the growth starting from the filamentous cell phase. At 36 hours the colonies developed from the coccoidal phase were very similar to those developed from the filamentous phase in about the same length of time. The observations on the growth obtained from the coccoidal phase were terminated at the end of 36 hours.

DISCUSSION

The direct observations on the multiplication of single cells clearly demonstrate that the coccoidal cells are pleomorphic forms of the actinomycetes studied. Coccoidal forms apparently occur more frequently among some strains of actinomycetes cultivatable from enamel surfaces than among the actinomycetes of Ørskov's Group IIb,[5] or among those elsewhere reported. The manner of sprouting and the initial divisions of the coccoidal phase, as observed in Strain 80, appear to be similar to these processes described in the coccoidal phase of the corynebacteriae and mycobacteriae.[5, 13, 14]

In other respects the morphology of the actinomycetes studied corresponds closely with those of Ørskov's Group IIb. This is in agreement with the observations of Sullivan and Goldsworthy,[15] who reported that their anaerobic oral actinomycetes belonged to Ørskov's Group IIb, and with Bibby and Knighton[16] who noted that their aerobic oral strains were similar to those of Sullivan and Goldsworthy. A characteristic feature is the manner in which cells of the filamentous phase divide into shorter bacillary cells lying at angles to one another in a zig-zag arrangement. The manner of branch formation by lateral budding and elongation of the bud resembles that reported to occur among other actinomycetes[6, 7] and also the corynebacteriae[17] and mycobacteriae.[18] Ørskov[5] and later Jensen[6, 7, 19] felt that in general the processes of cell multiplication and growth of Ørskov's group IIb actinomycetes were so similar to those of the corynebacteriae and mycobacteriae as to indicate a close taxonomic relationship between the three groups.

The resemblance which the pleomorphic oral strains may bear to the diphtheroids and cocci common among the oral flora complicates their identification. This difficulty applies not only to identification based on cell morphology but also to identification based on colonial morphology. The more anaerobic oral actinomycetes of the Wolff-Israel type which have been isolated from

tonsillar crypts, periodontal pockets, and carious detritus by numerous workers often change to intermediate or smooth colonies on prolonged artificial cultivation. As Rosebury[20] has pointed out, should such smooth colonies occur on primary isolation, they would be very difficult to distinguish from colonies of diphtheroids and staphylococci which they closely resemble.

The conditions which induce changes in cell or colonial morphology are not clear. Jensen[6] in an effort to determine the factors influencing the morphology of the soil actinomycetes concluded that while certain cultural conditions seemed to favor the development of particular cell and colony forms, these forms also developed spontaneously. The work of Ørskov,[5] Jensen,[6] and Sullivan and Goldsworthy[15] suggested that among the actinomycetes and mycobacteriae aging of the cultures tended to induce filamentous cells to divide into shorter cells. Age, per se, however, did not seem to be a determinant of cell length in the observations made on Cultures 80 and 85. Long filamentous cells of Culture 80 growing, but with little tendency to divide, were observed up to 120 hours of incubation in the microculture chamber while on agar slants complete transformation in cell phase sometimes occurred in only 24 hours. Since the changes in actinomycete morphology appear at present to be so unpredictable, it is not unreasonable to expect that they may occur in primary cultivation. This complicates any quantitative estimation of the actinomycetes cultivatable from tooth scrapings, for their recognition must depend upon metabolic and serologic characteristics, yet to be worked out, rather than on morphology alone.

As already mentioned, the pleomorphism of these organisms invites the hypothesis that coccoidal and filamentous organisms observed in the microscopic carious lesion may both be actinomycetes. This hypothesis gains some support from the observation that pleomorphic actinomycetes cultivated from enamel surfaces are capable of producing microscopic carieslike lesions in hamster enamel in vitro.[21, 22]

SUMMARY

In actinomycete cultures obtained from enamel surfaces, coccoidal cells were found to be intimately associated with the filamentous cells. Direct observation on cell division and growth showed that the coccoidal cells produced filamentous cells and were, therefore, pleomorphic forms. In other respects the morphology of these actinomycetes corresponded closely to those of Ørskov's Group IIb and to those of the Wolff-Israel type. The pleomorphism of these strains indicates that actinomycetes cultivated from enamel sometimes may be difficult to distinguish from the diphtheroids and cocci common among the oral flora. This pleomorphism further suggests that the filamentous and coccoidal forms observed histologically in preclinical carious enamel lesions may be actinomycetes.

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EFFECT OF CERTAIN HENDECYNOIC ACIDS AND THEIR AMMONIUM SALTS ON ACID PRODUCTION IN SALIVA CONTAINING GLUCOSE

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IT IS generally accepted that acid production in the mouth through the action of microorganisms plays an important role in the development of carious lesions. Thus, any reduction in acid formation, either through inhibition of the growth of acid-producing organisms or through interference with enzyme systems involved in acid production, should tend to prevent the formation of these lesions. Fosdick, Fancher, and Calandra[1] showed that 2-methyl-1,4-naphthoquinone markedly inhibited acid production in saliva-glucose solutions. Unfortunately this substance has an objectionable taste which is difficult to mask. Later, Fosdick and Calandra[2] reported on the inhibitory action of a variety of different substances, including organic peroxides, quinones, and various aldehydes. Kesel, O'Donnell, Kirch, and Wach[3] showed that a mixture of diammonium phosphate and urea markedly inhibited acid production and the growth of acid-producing organisms in saliva-glucose solutions. Dreizen and Spies[4, 5] found that products of protein putrifaction, such as indole and skatole, exert a marked inhibitory effect on the production of acid in saliva as well as on the growth of, and acid production by lactobacilli.

We have reported the considerable fungistatic action of the triple bond fatty acid, 9-hendecynoic acid ($CH_3C{\equiv}C(CH_2)_7COOH$), against a number of pathogenic fungi including those responsible for "athlete's foot."[6] Since this acid also exerted considerable inhibitory action against certain bacteria, it was decided to determine whether it would have an inhibitory effect on the rate of acid production in saliva containing glucose. Concomitant studies were carried out with the isomeric acids, 2-, 6-, and 10-hendecynoic acids, as well as with their ammonium salts. The triethanolamine salt was studied in the case of 9-hendecynoic acid but the results are not presented as they were very similar to those obtained with the ammonium salt. The solutions of the triethanolamine salt were prepared by melting the acid with the theoretical amount of triethanolamine, thoroughly mixing, and dissolving in water to give the desired concentration. These solutions were usually opalescent with pH around 7.2. Solutions of the ammonium salts were prepared by suspending the acid in water and adding concentrated ammonium hydroxide to dissolve it, with slight warming on a water bath. The pH of these solutions was around 8, except in the case of the 2-hendecynoic acid which was 5.5. All solutions were clear except that made from the 9-hendecynoic acid which was slightly opalescent.

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TABLE I

EFFECT OF FOUR ISOMERIC HENDECYNOIC ACIDS ON THE PRODUCTION OF ACID IN SALIVA CONTAINING GLUCOSE

EXPERIMENT NUMBER	INCUBATION PERIOD (HRS.)	SUBSTANCE USED	pH AFTER INCUBATION WITH THE INDICATED CONCENTRATION OF HENDECYNOIC ACID			
			70 MG. % (pH)	50 MG. % (pH)	25 MG. % (pH)	0 % (pH)
1	0	9-hendecynoic acid	--	7.2	7.3	7.3
	5	($CH_3C \equiv C(CH_2)_7COOH$)	--	6.1	6.1	5.4
	23		--	5.4	5.0	4.1
	96		--	4.9	4.6	3.9
2	0	9-hendecynoic acid	6.3	6.3	6.4	6.5
	24	($CH_3C \equiv C(CH_2)_7COOH$)	5.0	4.9	4.9	4.2
	48		5.0	5.0	4.9	4.1
	72		5.0	5.0	4.9	4.1
3	0	9-hendecynoic acid	6.5	6.7	6.8	6.9
	24	($CH_3C \equiv C(CH_2)_7COOH$)	4.9	5.0	5.0	4.3
	48		5.0	5.0	4.9	4.3
	72		5.0	5.0	4.9	4.3
4	24	9-hendecynoic acid	5.3	5.2	4.9	4.2
	48	($CH_3C \equiv C(CH_2)_7COOH$)	5.1	5.1	4.7	4.1
	72		5.1	5.0	4.7	4.0
5	0	10-hendecynoic acid	6.3	6.3	6.4	6.5
	24	($CH \equiv C(CH_2)_8COOH$)	5.0	5.1	5.1	4.2
	48		5.0	5.1	5.1	4.1
	72		5.0	5.0	5.0	4.1
6	0	10-hendecynoic acid	6.5	6.7	6.8	6.9
	24	($CH \equiv C(CH_2)_8COOH$)	5.1	5.0	5.0	4.3
	48		5.1	5.0	5.0	4.3
	72		5.1	5.0	5.0	4.3
7	0	10-hendecynoic acid	6.7	--	--	6.8
	24	($CH \equiv C(CH_2)_8COOH$)	5.0	5.1	5.2	4.3
	48		5.0	5.0	5.2	4.3
	72		4.9	5.0	5.1	4.3
8	0	6-hendecynoic acid	7.8	7.8	7.8	7.8
	24	($C_4H_9C \equiv C(CH_2)_4COOH$)	5.8	5.4	5.0	4.4
	48		5.1	4.7	4.2	3.9
	72		4.8	4.7	4.2	3.8
9	0	2-hendecynoic acid	7.2	7.3	7.4	8.2
	24	($C_9H_{19}C \equiv CCOOH$)	6.3	6.0	5.4	4.3
	48		5.1	4.7	4.8	4.0
	72		5.0	4.7	4.7	3.8

Effect of the various preparations on production of acid in saliva containing glucose.—Saliva from three individuals, collected with paraffin stimulation, was used throughout. After the saliva was collected, glucose was added to give a 5 per cent solution; 10 ml. portions of this saliva-glucose solution were transferred to test tubes and, when studying the effect of the free acid, weighed amounts of the acid were added to the requisite number of tubes. The tubes were thoroughly shaken to obtain complete solution. Two corresponding tubes without the hendecynoic acid served as controls. After determining the pH, the tubes were put in an incubator at 37°C., and their pH values measured at intervals thereafter, using a Beckman pH meter. Experiments using the

TABLE II

EFFECT OF SALTS OF FOUR ISOMERIC HENDECYNOIC ACIDS ON THE PRODUCTION OF ACID IN SALIVA CONTAINING GLUCOSE

EXPERIMENT NUMBER	INCUBATION PERIOD (HRS.)	SUBSTANCE USED	pH AFTER INCUBATION WITH SALTS AT THE CONCENTRATIONS INDICATED			
			100 MG. % (pH)	50 MG. % (pH)	25 MG. % (pH)	0 MG. % (pH)
1	0	Ammonium salt of	7.7	7.7	7.6	7.6
	24	9-hendecynoic acid	5.9	5.0	5.0	4.3
	48		5.1	4.9	4.2	4.0
	96		4.9	4.7	4.1	3.8
2	0	Ammonium salt of	7.7	7.7	7.6	7.5
	24	9-hendecynoic acid	5.4	5.4	5.1	4.3
	48		5.4	4.9	4.4	4.0
	96		5.2	4.9	4.2	3.8
3	0	Ammonium salt of	7.1	7.0	7.0	7.0
	24	9-hendecynoic acid	5.1	5.0	4.5	4.0
	48		5.1	4.9	4.4	3.9
	72		5.1	4.9	4.4	3.9
4	0	Ammonium salt of	7.6	7.6	7.5	7.5
	24	9-hendecynoic acid	5.1	5.0	4.5	4.2
	48		5.1	5.0	4.5	4.1
	72		5.1	5.0	4.4	4.0
5	0	Ammonium salt of	7.0	7.0	7.0	7.0
	24	10-hendecynoic acid	5.1	5.1	4.6	4.0
	48		5.0	5.0	4.6	3.9
	72		5.0	5.0	4.5	3.9
6	0	Ammonium salt of	7.5	7.5	7.5	7.5
	24	10-hendecynoic acid	5.2	5.0	4.9	4.2
	48		5.0	5.0	4.8	4.1
	72		5.0	5.0	4.7	4.0
7	0	Ammonium salt of	7.8	7.8	7.8	7.8
	24	6-hendecynoic acid	5.8	5.4	5.0	4.3
	48		5.1	4.7	4.2	3.9
	72		4.6	4.5	4.0	3.7
8	0	Ammonium salt of	7.4	7.4	7.4	7.4
	24	6-hendecynoic acid	5.6	5.3	5.0	4.2
	48		5.3	4.9	4.4	3.8
	94		5.1	4.9	4.3	3.7
9	0	Ammonium salt of	7.5	7.5	7.5	7.5
	24	2-hendecynoic acid	6.6	6.0	5.6	4.5
	48		6.1	4.5	5.0	3.8
	72		5.5	4.7	5.0	3.8
10	0	Ammonium salt of	7.7	7.7	7.7	7.8
	24	2-hendecynoic acid	6.7	6.0	5.5	4.4
	48		6.3	6.0	4.7	3.9
	72		5.0	4.4	4.5	3.6

ammonium and triethanolamine salts were carried out as above, except that the salts were added in the form of a 1 per cent solution, expressed in terms of the free acid. The final volume of each tube was kept the same by the addition of the appropriate amounts of water. The triethanolamine salt was used only in the case of 9-hendecynoic acid, since its activity did not seem to differ significantly from that of the free acid or its ammonium salt. The experimental results given in Tables I and II are typical of those obtained.

It will be seen from the results that the rate of acid production is materially reduced, in all cases, by concentrations as low as 25 mg. per 100 ml., expressed in terms of the free acid. 2-Hendecynoic acid showed greater inhibition of acid production during the first 24 hours than any of the others but by the end of 72 hours the pH had dropped as low as with the other preparations.

The inhibiting action of 9-hendecynoic acid and its ammonium and triethanolamine salts on acid production in saliva in the presence of glucose was confirmed in another way; 10 ml. samples of saliva-glucose solutions were transferred to 25 ml. Erlenmeyer flasks and 60 mg .of either finely ground dry bone or tricalcium phosphate were added. The flasks were incubated at 37°C. for about 20 hours, after which time they were shaken in a rotary shaker for 1 hour. The solutions were filtered and calcium was determined on a 5 ml. aliquot of the filtrate. The experimental results are shown in Table III. In general, the measurement of acid production by this method parallels that obtained by the pH method. In addition it demonstrates the inhibition by these substances of actual solution of bone salts or tricalcium phosphate under the conditions studied.

TABLE III

EFFECT OF 9-HENDECYNOIC ACID AND ITS SALTS ON THE SOLUTION OF CALCIUM FROM BONE OR TRICALCIUM PHOSPHATE IN A SALIVA-GLUCOSE SOLUTION

EXPERIMENT NUMBER*	SUBSTANCE USED	CALCIUM CONTENT AFTER INCUBATION WITH INHIBITOR AT CONCENTRATIONS INDICATED†					CALCIUM CONTENT OF ORIGINAL SALIVA
		100 MG. %	50 MG. %	25 MG. %	12.5 MG. %	0 MG. %	
1	Free acid	7.6	9.8	15.6	17.0	23.4	3.1
2	Free acid	--	8.3	15.6	16.2	17.5	4.4
3	Free acid	--	4.2	10.9	---	24.2	4.7
4	Triethanolamine salt	5.5	9.2	17.1	31.7	38.2	3.3
5	Triethanolamine salt	4.1	4.8	18.6	36.8	36.8	3.2
6	NH_4 salt	4.0	5.3	11.8	---	43.4	4.4
7	NH_4 salt	3.8	6.6	8.3	---	35.1	4.4

The concentrations of the salts are given in terms of the equivalent amount of the free acid.

Finely ground bone was used in Experiments 1 to 3, and tricalcium phosphate in Experiments 4 to 7.

*Saliva from only one individual was used for all these experiments.

†All calcium values are given in mg. per 100 ml.

The question naturally arises: Is this inhibition of acid production due to a reduction in the rate of bacterial growth or to an inhibiting action on some enzyme system involved in acid formation? In order to obtain information on the question, two experiments were carried out in which we studied the effect of various concentrations of 9-hendecynoic acid and of the ammonium salt on the growth of, and acid production by, a strain of *L. acidophilus* isolated originally from the oral cavity. The culture medium contained 1 Gm. proteose-peptone, 0.3 Gm. beef extract, 0.1 Gm. bacto-yeast extract and 1 Gm. glucose per 100 ml. of solution. Five tubes were used for each point on the curve. The pH of all tubes was adjusted to 7 after addition of the inhibitory substance and then autoclaved at 15 lbs. pressure for 20 minutes. After inoculation, they were incubated at 37° C. for 48 hours. Growth was measured turbidimetrically using a Klett-Summerson photoelectric colorimeter with a 42 filter.

Sterile control tubes carried through the same procedure served for the zero readings. The pH of each tube was determined after obtaining the turbidimetric reading. The experimental results are shown in Fig. 1. Similar results were given by both the acid and the salt. Growth inhibition and decreased acid production paralleled each other.

Effect on fermentation by baker's yeast (Fleischmann's).—In the course of the investigation it soon became evident that no yeast seemed to be growing in the saliva-glucose solutions containing the higher concentrations of inhibitor, while a fermentation odor was very evident in the control tubes. Since it has been suggested that yeast may work more or less in symbiosis with the acid-producing organisms in the production of carious lesions,[7] it was decided to determine the effect of 9-hendecynoic acid and its ammonium and triethanolamine salts on fermentation with baker's yeast. These substances had been shown to exert considerable fungistatic action on a number of pathogenic fungi.[6]

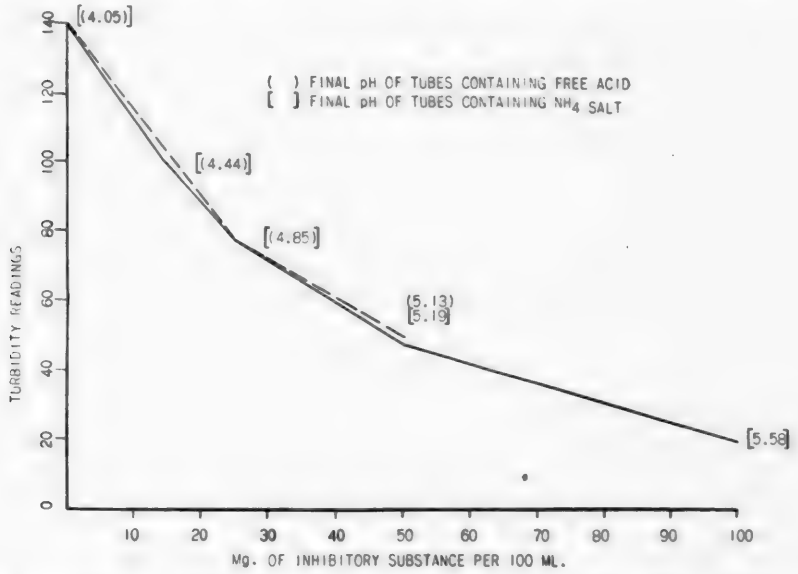

Fig. 1.—Growth of *Lactobacillus acidophilus* in the presence of different concentrations of 9-hendecynoic acid (broken line), or ammonium salt (solid line).

In these experiments, a solution of the free acid, or its salt, corresponding to the maximum concentration to be studied, was made in a 5 per cent glucose solution. It was necessary in the case of the free acid to warm the solution on a water bath in order to obtain an apparent concentration of 50 mg. per 100 ml. While the solution was still warm, measured amounts, 20, 19, and 5 ml., were transferred to test tubes and the volume in each case made up to 20 ml. with 5 per cent glucose in distilled water. The control tubes contained 20 ml. of this solution. As soon as the temperature of the various tubes had dropped to around that of the room, a weighed amount of baker's yeast was added to each and thoroughly dispersed throughout the solution. The contents

of each tube were transferred to fermentation tubes and incubated 37° C. for approximately 24 hours. The experimental results are shown in Table IV. The free acid is considerably more active in inhibiting yeast fermentation than either of its salts.

TABLE IV

EFFECT OF 9-HENDECYNOIC ACID, ITS AMMONIUM AND TRIETHANOLAMINE SALTS ON FERMENTATION BY BAKER'S YEAST

EXPERIMENT NUMBER	SUBSTANCE USED	PRODUCTION OF CO_2 IN PRESENCE OF INHIBITOR AT CONCENTRATIONS INDICATED					CONCENTRATION OF BAKER'S YEAST PER 100 ML (MG.)
		MG. % 100	50 MG. %	25 MG. %	12.5 MG. %	0 MG. %	
1	Free acid	--	0	0	++	++++	250
2	Free acid	--	0	0	++	++++	250
3	Free acid	--	0	0	+++	++++	250
4	Free acid	--	0	0	+++	++++	250
5	Free acid	--	0	+++	++++	++++	500
6	Free acid	--	0	+++	++++	++++	500
7	Triethanolamine salt	0	+	++	-----	++++	250
8	Triethanolamine salt	0	+	++	-----	++++	250
9	NH_4 salt	+	+	++	+++	++++	250
10	NH_4 salt	+	+	++	+++	++++	250

Amount of CO_2 produced indicated by + signs.

It would appear from the results obtained that studies on the possible use of these and related substances in dentifrices as possible agents in the prevention of dental caries would be justified. Toxicity studies with 9-hendecynoic acid and its ammonium and triethanolamine salts on both rats and mice indicate that they are relatively nontoxic.

SUMMARY

2-, 6-, 9-, and 10-Hendecynoic acids have been shown to exert considerable inhibitory action on the production of acid in saliva containing glucose. A similar effect was shown by their ammonium and other salts studied. 9-Hendecynoic acid exerts a considerable inhibitory action on fermentation by baker's yeast. Its ammonium and triethanolamine salts, although active, show considerably less activity in this regard.

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THE PRODUCTION OF ACIDS FROM GLUCOSE BY ORAL MICROORGANISMS: CITRIC ACID

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THE VARIOUS acids produced by the action of oral microorganisms on glucose will always be of interest in connection with the dental caries problem. It is well known, of course, that a variety of organic acids, such as lactic acid, pyruvic acid, acetic acid, propionic acid, butyric acid, etc., are end products of the action of various microorganisms on glucose. In connection with salivary microorganisms, it has long been known that lactic acid results from their action on carbohydrates. More recent studies by Summerson and Neuwirth[1, 2] have shown that lactic and pyruvic acids do not account for all of the acid resulting from the fermentation of glucose by oral microorganisms under physiological conditions. Fosdick[3] reported the isolation of lactic, pyruvic, phosphoglyceric, and possibly butyric and hexosephosphoric acids following a two-week incubation of a saliva-glucose-tribasic calcium phosphate mixture. Working under somewhat more physiological conditions, Muntz[4] reported finding, besides lactic acid, acetic, propionic, and formic acids from the incubation of glucose with dental plaque material.

In this paper a study of the possible production of citric acid from glucose by oral microorganisms is reported. Because of the well-known ability of citric acid to form soluble complexes with calcium, such a study is of importance in connection with tooth decalcification.

TABLE I

CITRIC ACID DETERMINATIONS IN INCUBATED GLUCOSE-SALIVA MIXTURE*

	EXPERIMENT NO.	AGE	GLUCOSE MG. PER CENT INCUBATION		CITRIC ACID γ PER CENT INCUBATION	
			BEFORE	AFTER	AFTER	BEFORE
Group I*	2	21	172	102	272	121
	3	18	154	84	252	161
	4	21	150	66	169	83
	5	26	165	140	611	198
	6	24	153	113	321	121
	11	23	147	69	335	269
	14	40	150	96	183	85
Group II†	1	24	159	84	355	83
	7	21	149	98	124	56
	8	23	151	90	580	117
	9	21	147	92	183	127
	13	23	153	122	534	129

*One per cent glucose added to saliva to give an approximate glucose concentration of 150 mg. per cent. The incubation period was 30 minutes.

†Group I subjects are caries susceptible, but showed no caries at time of experiment. Group II had active caries at time of experiment.

All subjects are males.

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TABLE II

CITRIC ACID DETERMINATIONS IN SALIVA-GLUCOSE MIXTURE* AFTER VARYING TIMES OF INCUBATION

EXPERIMENT NO.	AGE AND SEX	GLUCOSE MG. PER CENT INCUBATION TIME IN MINUTES									CITRIC ACID γ PER CENT INCUBATION TIME IN MINUTES								
		0	10	15	20	30	40	45	50	60	0	10	15	20	30	40	45	50	60
16	24M†	174	---	143	---	134	---	125	---	116	815	---	597	---	343	---	234	---	163
18	20F†	138	---	120	---	101	---	87	---	75	137	---	38	---	38	---	28	---	28
20	25M‡	165	125	---	108	90	75	---	70		240	87	---	57	42	42	---	32	---
24	54M§	135	130	---	114	108	100	---			1030	808	---	528	262	134	---		

*One per cent glucose added to saliva to give an approximate glucose concentration of 150 mg. per cent.
†Caries active.
‡Caries inactive; susceptible.
§Caries immune.

EXPERIMENTAL AND RESULTS

Saliva was collected in a small beaker 2 to 3 hours after the last meal, usually breakfast. As a rule, it took from 5 to 10 minutes to collect 10 ml. of saliva. The subject was given a small piece of paraffin (10 × 10 × 15 mm.) with instructions to chew it vigorously and to shift it about in the mouth in order to dislodge organisms from the teeth.

Glucose was added to the saliva and incubation allowed at 37° C. for the periods indicated in Tables I and II. Glucose was determined by a micromodification of the Folin-Wu method[5] and citric acid by the Natelson-Pincus-Lugovoy procedure.[6]

Table I shows that regardless of the caries condition, oral microorganisms act on glucose without the production of citric acid. On the contrary, during the 30-minute experimental incubation, there is a definite loss of the original citric acid present in the saliva.

Table II represents similar experiments in which determinations of glucose and citric acid were made at varying short time intervals of incubation. In no instance were we able to detect the production of citric acid from the action of oral microorganisms on glucose in as short an interval as 10 or 15 minutes.

TABLE III

ACTION OF ORAL MICROORGANISMS ON ADDED SODIUM CITRATE SOLUTIONS

EXPERIMENT NO.	AGE AND SEX	CARIES CONDITION	CITRIC ACID γ PER CENT TIME OF INCUBATION IN MINUTES								
			0	10	15	20	30	40	45	50	60
21	23M	Active	2450	---	---	---	770				
19	20F	Active	1200	---	430	---	132	---	13	---	0
27	6M	Active	1620	618	---	408	195	172			
32	10M	Active	1480	1100	---	885	735	525			
17	23M	Inactive, susceptible	943	---	580	---	173	---	87	---	66
22	23M	Inactive, susceptible	1660	1490	---	1270	912	575	--	367	214
28	30M	Inactive, susceptible	1520	930	---	855	570	438			
25	54M	Immune	2330	2200	---	1910	1410	980			

In Table III it is shown that added citrate is rapidly utilized by oral microorganisms. This is also true of the citrate originally present in the saliva, as is evident from Table IV. This was also found and noted in the experiments reported in Tables I and II. The rapid disappearance of citrate from saliva has also been shown by Zipkin and McClure.[7]

TABLE IV

THE DISAPPEARANCE OF CITRATE FROM SALIVA

EXPERIMENT NO.	AGE AND SEX	CARIES CONDITION	CITRIC ACID γ PER CENT TIME OF INCUBATION IN MINUTES					
			0	10	20	30	40	50
26	24M	Active	725	480	274	153	87	78
30	9M	Active	248	208	196	126	89	
31	20F	Active	564	340	300	256	168	
15	40M	Inactive, susceptible	222	---	---	97		
23	18M	Inactive, susceptible	673	458	303	162	100	94
29	30M	Inactive, susceptible	313	234	209	150	150	

SUMMARY

Citric acid is not produced from the action of oral microorganisms on glucose. Indeed, it seems that added citrate, or that originally present in saliva, is rapidly utilized by oral microorganisms.

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THE INHIBITION OF ACID FORMATION IN THE MOUTH BY AMINO DERIVATIVES OF 2,3-DICHLORO-1,4-NAPHTHOQUINONE

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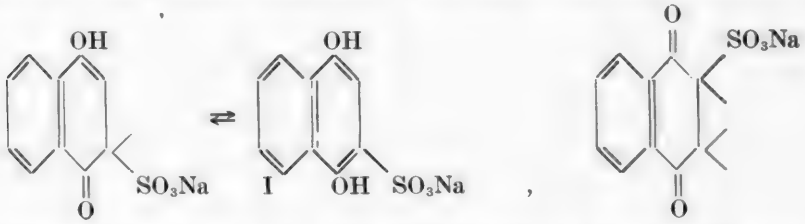
IT HAS been shown that compounds of the naphthoquinone type inhibit acid formation in the mouth.[1, 2] The mechanism of inhibition is probably the tying up of sulfhydryl groups of enzymes that are involved in the acid formation. This would mean a competitive inhibition between the naphthoquinone and the substrate for the enzyme. The nature of the addition product of the enzyme and the quinone has not been demonstrated, but it may be similar to that of the bisulfite addition product of menadione. The latter product has two possibilities:



Carmach, Moore, and Balis[3] by their spectrophotometric studies have shown the second structure to be the compound effective as an antihemorrhagic. The sulfhydryl enzymes could easily form a reversible complex with the naphthoquinones in the same manner.

It was desired to test the inhibition properties of a series of amine derivatives of 2,3-dichloro-1,4-naphthoquinone. The amines were of four types: aminopyridines, sulfonamides, alkylamines, and amino acids. The inhibition tests were made in the usual manner using the free calcium as an index of acid formation.[1] The results are shown in Table I. The synthesis of the compounds will be reported elsewhere.

The inhibition results were reported in part at the meeting of the Chicago Section of the International Association for Dental Research, June, 1949.

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TABLE I

INHIBITOR	AMOUNT ADDED MG./100 C. C.	MILIMOLES/LITER	CALCIUM MG./100 C. C.
3-(2-Chloro-1,4-naphtho-quinonyl)-N-sulfapyri-dine	0	0	19.8
	4	0.09	11.0
	8	0.18	5.1
	12	0.24	3.1
	16	0.36	2.4
	20	0.45	2.9
	24	0.54	4.4
	50	1.14	2.0
3-(2-Chloro-1,4-naphtho-quinonyl)-N-sulfanila-mide	0	0	19.8
	50	1.38	10.3
3-(2-Chloro-1,4-naphtho-quinonyl)-N-sulfadia-zine	0	0	21.8
	4	0.09	5.4
	8	0.18	3.9
	12	0.27	3.2
	16	0.36	3.1
	20	0.45	2.1
	24	0.54	3.7
	50	1.14	2.4
3-(2-Chloro-1,4-naphtho-quinonyl)-N-sulfathia-zole	0	0	21.8
	12	0.27	5.7
	16	0.36	2.6
	20	0.45	3.4
	24	0.54	2.9
	50	1.12	2.0
3-(2-Chloro-1,4-naphtho-quinonyl)-N-sulfamera-zine	0	0	25.4
	50	1.14	2.2
3-(2-Chloro-1,4-naphtho-quinonyl)-N-sulfa-[3-(2-chloro-1,4-napthо-quinonyl)-N′-guani-dine]	0	0	19.8
	12	0.21	15.3
	16	0.28	14.4
	20	0.35	15.6
	24	0.42	2.6
	50	0.88	10.0
3-(2-Chloro-1,4-naphtho-quinonyl)-N-guanidine carbonate	0	0	19.8
	16	0.31	20.0
	20	0.38	11.2
	24	0.46	10.0
	50	0.96	3.4
2-Chloro-3-di-*n*-propyl-amino-1,4-naphthoqui-none	0	0	21.3
	24	0.82	15.9
	50	1.72	10.1
2-Chloro-3-*n*-decylamino-1,4-naphthoquinone	0	0	24.2
	50	1.44	18.7
3-(2-Chloro-1,4-naphtho-quinonyl)-N-2-amino-pyridine	0	0	24.4
	12	0.44	14.1
	16	0.59	12.2
	20	0.73	10.4
	24	0.88	8.2
	50	1.83	4.0
3,3′-*bis*-(2-Chloro-1,4-naphthoquinonyl)-N-2-amino-3-methylpyridine	0	0	24.4
	12		22.4
	16		19.9
	20		15.6
	24		14.3
	50		4.5

TABLE I—CONT'D

INHIBITOR	AMOUNT MG./100 C. C.	ADDED MILIMOLES/LITER	CALCIUM MG./100 C. C.
3-(2-Chloro-1,4-naphtho-quinonyl)-N-2-amino-5-methylpyridine	0	0	17.8
	8	0.16	2.5
	12	0.25	3.3
	24	0.49	3.8
	50	1.02	4.0
3-(2-Chloro-1,4-naphtho-quinonyl)-N-amino-caproic acid	0	0	23.8
	24	0.82	17.0
	50	1.70	11.3
3-(2-Chloro-1,4-naphtho-quinonyl)-N-4-amino-*iso*-phthalic acid	0	0	17.8
	5	0.13	10.4
	10	0.27	6.0
	25	0.67	4.1
	50	1.34	3.5

RESULTS

The results shown in Table I indicate that all of the compounds are effective to some extent. The sulfonamides appear to be the most effective. Probably here the sulfonamide structure as well as the naphthoquinone is concerned in the inhibition. The pyridine derivatives, while very effective at the maximum concentration used, do not inhibit in as low concentrations as the sulfonamides. The two amino acid derivatives appear to be the least effective.

SUMMARY

The inhibition by amine derivatives of 2,3-dichloro-1,4-naphthoquinone of acid formation in saliva is presented. The greatest inhibition is obtained with the sulfonamide derivatives.

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INVESTIGATIONS ON THE METABOLISM OF FLUORIDE

II. Fluoride Content of Blood and Urine as a Function of the Fluorine in Drinking Water

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INTEREST in the physiological aspects of fluorine has grown rapidly since the identification of this element as the causative agent in dental fluorosis or mottled enamel. Additional impetus has been supplied by the several studies currently in progress on the control of dental caries by fluorination of a community water supply. The urinary excretion of fluoride has now become a generally accepted criterion for the estimation of fluorine exposure and absorption. Ample evidence for the validity of this criterion is to be found in the investigations of Brun, Buckwald, and Roholm on fluoride poisoning in cryolite workers,[1] and in those of Largent and Ferneau on workers in magnesium foundries,[2] and in the manufacture of high octane gasoline.[3] Of the numerous investigations into the urinary excretion of fluoride by normal individuals, that of McClure and Kinser[4] is the most extensive. In this study, it was shown that a mathematical relationship existed between the concentrations of fluoride in the drinking water and in the urine.

Because fluoride is excreted principally via the urine[5] it follows that the fluoride is necessarily transported to the kidney by means of the circulating blood. It is logical to inquire then, whether or not an increase in the fluoride content of a community water supply to a level of approximately 1 ppm will result in a parallel increase in blood fluoride content. The investigation to be described was designed to answer this question.

EXPERIMENTAL

Blood and urine samples for this study were obtained from volunteers residing in the two towns of Rochester and Newburgh, N. Y.* The water supply of the former community contains approximately 0.06 ppm F while that of Newburgh contains 1.0 to 1.2 ppm F.[6] Because it was possible to obtain only nonfasting blood samples from the Newburgh residents, nonfasting samples from the Rochester population were used for comparison. However, a series of fasting samples were also taken from the Rochester volunteers in order to determine the effect of food consumption on the blood fluoride content. Concurrent spot urine specimens were also obtained from the Newburgh residents; overnight urine samples were collected by the Rochester donors.

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*The Newburgh samples were obtained from personnel of the duPont Fabrikoid plant, Newburgh, N. Y.

The blood fluoride concentrations were determined by the method of Smith and Gardner.[7] The urine specimens were prepared for analysis and the fluoride removed by distillation according to the method described by Flagg;[8] the fluoride content of the distillates was determined by the titration procedure described by Smith and Gardner. The results described below are based upon work reported in the Quarterly Technical Report of the University of Rochester Atomic Energy Project.[9]

RESULTS

The distribution of the fluoride content of the blood samples is shown in Table I. Inspection of these data shows that 45 per cent of the nonfasting Rochester samples contained no detectable fluoride. In contrast, an equivalent percentage (42 per cent) of the Newburgh samples contained 3 to 6 μg F/100 ml. Only 1 sample of those obtained from Newburgh residents contained no fluoride (less than 0.5 μg F/100 ml.). Because 88 per cent of the fasting Rochester samples contained no measurable quantities of fluoride, it is apparent that the ingestion of food 2 to 3 hours prior to the taking of the sample produces slightly higher blood fluoride contents in approximately one-half of the volunteers. A statistical analysis of the difference between the means of the fasting and nonfasting Rochester blood samples (Table III) indicates that the difference (1.05 μg F/100 ml. blood) could only be due to chance in 2 of 100 instances. It is also evident that the ranges of nonfasting blood fluoride contents were identical for Rochester and Newburgh.

TABLE I

DISTRIBUTION OF FLUORIDE CONTENT OF NORMAL HUMAN BLOOD SAMPLES FROM ROCHESTER AND NEWBURGH, N. Y.

μG F PER 100 ML. BLOOD	ROCHESTER*				NEWBURGH†	
	FASTING		NONFASTING		NONFASTING	
	NO. SAMPLES	PER CENT	NO. SAMPLES	PER CENT	NO. SAMPLES	PER CENT
0.0	30	88	9	45	1	8
0.4	1	3	3	15	0	0
1.0 to 2.0	1	3	3	15	2	17
2.0 to 3.0	0	0	2	10	2	17
3.0 to 6.0	2	6	2	10	5	42
6.0 to 9.0	0	0	1	5	2	17
Totals	34	100	20	100	12	101

*Water supply contains 0.06 ppm F.
†Water supply contains 1.36 ppm F.

In Table II is shown the distribution of the urinary fluoride concentrations. Seventy-seven per cent of the Rochester overnight samples are free of fluoride whereas appreciable quantities of fluoride were found in all of the Newburgh samples. The 2 groups of samples overlap in fluoride content at the 0.4 to 0.5 mg. F/liter range which includes only the highest 10 per cent of the Rochester samples while the lowest 17 per cent of the Newburgh samples fall at this range. The range of the fluoride contents of the Newburgh samples is 0.4 to 2.4 mg. F/liter, with an average content of 1.12 mg. F/liter. This mean concentration is in excellent agreement with the fluoride content of the water supply of this

TABLE II

DISTRIBUTION OF FLUORIDE CONCENTRATIONS OF NORMAL HUMAN URINE SAMPLES FROM ROCHESTER AND NEWBURGH, N. Y.

MG. F PER LITER URINE	ROCHESTER		NEWBURGH	
	NO. SAMPLES	PER CENT	NO. SAMPLES	PER CENT
0.0	23	77	0	0
0.1	4	13	0	0
0.4	2	7	1	8.3
0.5	1	3	1	8.3
0.7			1	8.3
0.8			1	8.3
0.9			1	8.3
1.0			1	8.3
1.1			1	8.3
1.2			1	8.3
1.3			1	8.3
1.5			1	8.3
1.7			1	8.3
2.4			1	8.3
Totals	30	100	12	100

community (1.0 to 1.2 ppm) and in reasonable agreement with the urinary fluoride content predicted (0.95 ppm F) for this water concentration from the data of McClure and Kinser.[4]

A sample of the Newburgh water supply taken the day after the blood and urine specimens were collected, was found to contain 1.36 ppm. F. This concentration is considered to agree, within the limits of precision of the analytical method,[7] with the concentration of 1.0 to 1.2 ppm F reported by Ast, Finn, and McCaffrey[6] for Newburgh water. The data of Ast et al. are based upon repeated analyses over a prolonged period of time.

TABLE III

T-RATIOS FOR THE SIGNIFICANCE OF THE DIFFERENCES IN MEANS OF BLOOD AND URINARY FLUORIDE CONCENTRATIONS OF THE POPULATIONS OF ROCHESTER AND NEWBURGH, N. Y.*

VARIABLE	GROUP†	NO. OF SAMPLES	MEAN	STANDARD DEVIATION	DIFFERENCE IN MEANS	T-RATIO	P-MEANS
μg F/100 ml. blood	A	34	0.33	1.16	1.05	2.29	0.02
	B	20	1.38	2.22			
	A	34	0.33	1.16	3.60	6.94	0.01
	C	12	3.93	2.35			
	B	20	1.38	2.22	2.55	3.08	0.01
	C	12	3.93	2.35			
mg. F/liter urine	A	30	0.06	0.13	1.06	9.91	0.01
	C	12	1.12	0.56			

*The statistical analyses were performed by Mrs. Helen Michaels of the Statistics Section, University of Rochester Atomic Energy Project.

†A—Rochester fasting samples.
B—Rochester nonfasting samples.
C—Newburgh nonfasting samples.

In Table III is presented a statistical analysis of the differences in mean blood and urine fluoride levels for Rochester and Newburgh populations. The average blood fluoride content of the Newburgh population is greater than that

of Rochester, and is a result of the addition of fluoride to the Newburgh water supply. The value of 2.6 μg F/100 ml. noted for the difference in means of the Newburgh and Rochester blood samples of nonfasting donors could be the result of chance in less than 1 time in 100 such comparisons. Such conclusions must be obvious in this instance, inasmuch as 88 per cent of the Rochester samples from fasting individuals contained no demonstrable quantities of fluoride. As would be expected on the basis of the work of McClure and Kinser,[4] the mean urinary fluoride concentration of the Newburgh residents is significantly higher than that of the Rochester residents. This difference of 1.06 mg. F/liter is certainly attributable to the greater concentration of fluoride in the Newburgh water supply.

TABLE IV

SUMMARY OF MEAN NONFASTING BLOOD AND URINE FLUORIDE CONCENTRATIONS OF RESIDENTS OF ROCHESTER AND NEWBURGH, N. Y.

SAMPLE	FLUORIDE CONCENTRATION, PPM F	
	ROCHESTER	NEWBURGH
Water supply	0.06	1.36
Urine	0.06	1.12
Blood	0.014	0.040

Table IV presents a concise summary of the results on drinking water, urine, and blood obtained in this study. To permit immediate comparisons, all values are expressed as parts per million of fluoride. It is seen that the mean water and urine fluoride concentrations are in excellent agreement for each community. The fluoride concentrations for the urine and blood of the Newburgh residents are significantly greater than the corresponding values for those residents of Rochester; these increases are attributed to the greater concentration of fluoride in the Newburgh water supply. It will be noted that while a nineteen fold increase has occurred in the mean urinary fluoride concentration of the Newburgh population, the blood fluoride concentration of these individuals has increased only threefold. While none of the Newburgh bloods were free of fluoride (Table I), the maximum concentration found did not exceed that found in the highest nonfasting Rochester samples. Moreover, the concentrations found are all of an extremely low order of magnitude. Apparently, at the level of approximately 1 ppm of fluoride in the drinking water, the normal individual is capable of adequately excreting the ingested fluoride, thereby preventing the accumulation of toxic quantities of fluoride in the blood.

To date, toxic effects in man have not been associated with concentrations of blood fluoride of the magnitude encountered here. Gettler and Ellerbrook[10] found 0.35 to 1.55 mg. of F/100 ml. of blood in 5 fatal cases of acute sodium fluoride poisoning. These concentrations are approximately thirty-five to one hundred and fifty-five fold greater than those obtained in the Rochester-Newburgh study. Wilkie[11] has reported 2 cases of osteosclerosis in fluoride workers, with accompanying blood and urine fluoride determinations. One individual employed 3½ years in the aluminum industry (exposure to cryolite) showed 299 μg F/100 ml. of blood and 1.73 mg. F per 24-hour urine volume.

The second individual was employed in the manufacture of hydrogen fluoride for 16 years; corresponding blood and urine fluoride concentrations were 98 μg F and 15.22 mg F, respectively. Normal values reported by Wilkie were 47 μg F/100 ml. blood and 1.32 mg. F. per 24-hour urine volume. While Wilkie's blood fluoride concentrations appear to be somewhat higher than were found in the Rochester-Newburgh study, it is nevertheless evident that a considerable margin of safety exists between the concentrations of fluoride in blood found in Rochester and Newburgh residents, and blood levels that might be associated with the appearance of osteosclerosis.

SUMMARY

A comparison has been made of the blood and urinary fluoride concentrations in 2 populations whose water supplies contain 0.06 and 1.36 ppm fluoride, respectively. The data indicate:

1. As the fluoride concentration of the water supply is increased from 0.06 to 1.36 ppm (twenty-three fold increase), the mean urinary fluoride concentration increases from 0.06 to 1.12 ppm (nineteen fold increase).

2. Under these same conditions, the mean blood fluoride concentration increases from 0.014 to 0.040 ppm (threefold increase).

3. A maximum blood fluoride concentration of 0.1 ppm was found in each population. It is, therefore, concluded that the higher level of fluoride in the water supply does not produce dangerously high blood fluoride concentrations.

We are pleased to acknowledge the cooperation of Dr. C. E. Fallon of the duPont Fabrikoid plant, and Drs. David E. Overton and Sidney B. Finn of the Newburgh Fluoride Demonstration Project in obtaining the blood and urine samples from that community. We are also indebted to Dr. J. Howland for taking the Rochester blood samples, and to the residents of both communities who contributed the samples needed. Mrs. Dorothy Wing and Miss Pearl Dunn assisted with the analyses.

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VIBRATION CHARACTERISTICS OF THE ROTATING DENTAL INSTRUMENT

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INTRODUCTION

PATIENT discomfort in restorative dentistry has been attributed mainly to the two factors of heat generation and the vibration produced during the cutting operation on the tooth. A review of the literature, however, gives little information on either the extent or chief source of vibrations.

Gabel reported that the chisel produced the least pain of any instrument used to cut dentin. While the reason for this is not clear, Gabel speculated that it is due in part to the lack of vibration and noise and to a small heat production.[1] Braceland[2] referred to the work of Best,[3] in which he described the relationship of vibration to pain, and pointed out that the dental engine frequently starts painful, annoying stimuli to bring about a reaction of aversion on the part of the patient.

In a direct attempt to measure the nerve reaction set up by vibratory stimuli applied to the teeth, Pfaffman made certain interesting observations.[4] Electrically controlled sinusoidal waves, checked oscillographically, were applied to cats' teeth. Nerve endings were placed between plates and the nerve response recorded on the oscillograph. "Spikes" resulted as a nervous reaction on the oscilloscope, which continued after the stimulus was removed. The nerve responded faster and more intensely to high frequencies, but the nervous reaction died faster than the response to low frequency. When the initial shock was over, the nerve set up a vibration frequency identical with the impulse. This observation is of some interest in the study of vibrations produced by the rotating dental instrument.

The work reported by Walsh and Symmons[5] concluded that (1) the upper limit of vibration perception is about 600 C.P.S., (2) a region of "maximum unpleasantness" exists between 150 C.P.S., and 200 C.P.S. and (3) the dental engine and handpiece produce a dominant vibration at about 100 C.P.S. Their first two conclusions are interesting in that they limit the field of vibration that should be studied. The third conclusion was arrived at from data obtained from patient reaction and from values obtained by using a rigidly mounted handpiece to cut away the surface of a brass slug, mounted in a vertical cylinder.

These references indicate that vibration and noise are a source of patient discomfort and show that only little work has been done on research in any aspect of the subject of vibrations resulting from dental operations.

It can be demonstrated that the vibrations produced by the rotating dental bur are in part a function of the material being cut, and the harder the

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material, the higher the harmonic content of the vibration. The choice of the material to be cut and the method of mounting the sample, therefore, is of primary importance in laboratory studies of this type. It is suspected that only relative or comparative values can be obtained from measurements on any material other than natural teeth supported by the tissues of the mouth.

It should be stated, therefore, that the results being reported at this time were produced under the laboratory conditions stipulated, and should be considered as typical values for the condition under which the test was made. This study should be considered as a comparison of vibrations produced by various instruments, and only as an indication of the vibrations experienced when cutting teeth in the mouth. Studies have been initiated in which measurements are being recorded of vibrations resulting from typical cavity preparations in teeth in the mouth.

PURPOSE OF STUDY

It was the purpose in this study to examine in a comparative fashion the vibrations produced by prevailing standard dental equipment, using various types of accepted cutting instruments, operated at speeds and applied pressures that are varied over the usual practical operating range for such equipment. No effort has been made to include measurements on equipment operated at exceptionally high speeds or other experimental conditions which would involve equipment of a special design. Only typical values are being reported at this time and no effort is made to report in detail all data thus far obtained. Some general correlations have been observed between the heat generated and the vibrations produced during the cutting operation. The results obtained from a study made of the vibration characteristics resulting from the use of the gas-propelled abrasive in cavity preparation will be described briefly.

METHOD OF TEST

The vibrations reported are those produced in a block of ivory (of 1 cm. square cross section and 5 cm. long) attached to the platform of an ordinary spring balance scale. The handpiece was pivoted on a knife edge behind the knurled portion of the sheath, with the side of the bur resting on the top of a block of ivory. The engine and handpiece were operated at three different speeds in both the forward and reverse directions for each of five weights applied to the handpiece. The operating speeds were developed by making divisions at approximately one-third of the arc of movement of the foot control for the engine, in each direction from the neutral position. The measured prevailing speeds were approximately 1100, 2300 and 3800 r.p.m. Both forward and reverse directions of operation were studied to determine certain fundamental characteristics of operation which are not revealed by operating in one direction only. The weights applied were 200 Gm., 500 Gm., 1 kg., 1½ kg., and 2 kg., which gave a load of approximately ¼ lb., ¾ lb., 1½ lbs., 2 lbs., and 3 lbs. on the spring balance scale.

A Pickering magnetic reluctance type phonographic pick up was placed in a record player tone arm with the needle of the pick up resting on the ivory

block. In this pick up was produced an electrical voltage which when calibrated for various responses has an amplitude-time relationship corresponding to the vibration in the ivory block.

This voltage was amplified and led to a cathode-ray oscilloscope, giving a visual record which was then photographed. The photographed trace was projected on a screen and amplified until the projected period of oscillation, or pattern, was 40 cm. wide. This projected trace pattern was analyzed through the use of a Coradi analyzer. The analyzer gives the amplitudes of sine waves at the fundamental frequency and at frequencies that are multiples of the fundamental frequency. When superimposed upon each other these waves produced the complex vibratory wave. (This type of analysis is known by the name Fourier analysis or Fourier series analysis.) In the beginning of this work a General Radio sound analyzer (Type 760A—serial 161) was connected electrically in parallel with the oscilloscope. To correlate theoretical analysis with actual vibrations, an unloaded handpiece, a handpiece using a round bur, fissure bur, and carborundum stone, were studied when cutting a block of ivory. It was found throughout this initial study that the vibration components are in harmonics validating the form of analysis that was used.

In order to make direct comparisons of different operating conditions with regard to the frequencies and amplitudes of the various fundamental and harmonic waves observed, a photograph of a sine wave of known frequency and amplitude was taken immediately after the trace of the vibratory wave. For this sine wave the oscilloscope control settings were identical with the settings used for the vibratory wave. The photographed oscillator trace (sine wave) was also projected and the amplitude measured. The amplitudes of the fundamental and harmonic waves presented in the tables of this report are the readings obtained from the Coradi analyzer as measured in centimeters, and modified or related to a common projected oscillator trace of 25 cm. amplitude.

VIBRATIONS OBSERVED

In Table I are shown the components of the vibrations of the handpiece alone, which show the side-to-side oscillations of the handpiece at the collet end. To determine these vibrations, the handpiece was wrapped in sponge rubber to resemble the resiliency of the hand grip, and then held in a clamp near the end of the knurled portion of the sheath, opposite to the collet. The needle of the

TABLE I

OPERATING CONDITION*	FUNDAMENTAL FREQUENCY C.P.S.†	CALCULATED SPEED OF ROTATION R.P.M.	FUNDAMENTAL AMPLITUDE (RELATIVE)	AMPLITUDES OF HARMONICS			
				2	3	4	5
3F	50	3,000	3.68	1.70	0.58	0.15	0.24
2F	36	2,160	3.22	0.17	0.57	0.38	0.05
2R	36	2,160	3.63	0.99	0.16	0.18	0.09

*3F = 3810 r.p.m. forward.
2F = 2139 r.p.m. forward.
2R = 2175 r.p.m. reverse.
†C.P.S. = cycles per second.

pick up was placed in contact with the sheath at the collet end, in such a way as to record the sidewise oscillations. No data are presented to show the end-thrust vibrations of the handpiece. The operating conditions noted are 3F (Speed 3, forward), 2F, and 2R (Speed 2, reverse).

It can be seen from this table that the fundamental frequency of vibration in cycles per second changes with operating speed. It is likewise observed that the fundamental vibration wave occurs at the speed of rotation of the handpiece shaft, as shown by multiplying the fundamental frequency by 60. These data indicate also that a portion of the vibrations observed when cutting with a bur may in part be produced either by the engine, transmitted through the metal arm, or by the rotation of the handpiece shaft, and magnified by the cutting action of the bur.

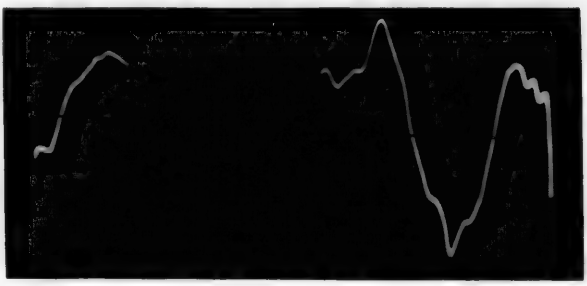

Fig. 1.—Character of vibration wave produced by handpiece alone, Speed 2, forward.

From Table I it is observed also that the amplitude of the fundamental vibration is larger than any harmonic, by a ratio of at least 2 to 1, and that the relative amplitudes of the harmonics decrease rapidly as they are extended beyond the second. The data presented here are for the first five harmonics, although calculations have been carried to the tenth. As will be seen in subsequent figures, the amplitudes of the sixth to tenth harmonics are small. The largest amplitude value of the fundamental vibration is 3.68 for Speed 3 operated in the forward direction. The character of this wave is shown in Fig. 1.

An additional source of vibration which has been observed is that produced by the splice in the engine belt. While no effort has been made to analyze in detail the vibration from this source, it has been detected and recorded in these studies by a characteristic shape of the vibration curve which is repeated at definite intervals. The effect was most pronounced at approximately 2,100 r.p.m., at which speed it can be shown to occur once in each 1.26 seconds, when using a belt 128 inches long on a handpiece pulley of approximately one inch in diameter.

THE EFFECT OF DIFFERENT BURS

The results obtained from the study of five burs, including the No. 557 fissure bur, No. 37 and No. 39 inverted cone burs, and No. 2 and No. 4 round burs are shown by the values given in Tables II, III, and IV. In Fig. 2 is shown the relative amplitude of the various harmonics to the tenth, for all five burs

operated at speed 3F (approximately 3,800 r.p.m.) and a pressure of 1½ lbs. Values are plotted in Fig. 3 to show the relative amplitude of the same harmonics resulting from all five burs operated at speed 2F (approximately 2,300 r.p.m.)

TABLE II

BUR	OPERATING CONDITION*	LOAD IN GRAMS	FUNDAMENTAL FREQUENCY	CALCULATED SPEED OF ROTATION	FUNDAMENTAL AMPLITUDE	AMPLITUDES OF HARMONICS			
						2	3	4	5
557	2R	200	25.6	1,535	9.15	0.78	1.67	0.37	0.41
	2F		—	2,560	9.22	1.72	1.41	0.74	1.36
	3F		—	3,460	2.01	2.28	1.55	0.75	0.28
557	2R	500	24.4	1,465	2.48	3.25	2.26	1.05	0.81
	3F		42.7	2,560	12.7	2.2	1.13	0.56	0.35
557	2R	1,000	24.4	1,465	9.0	0.43	2.6	0.64	0.34
	2F		19.8	1,190	8.33	1.28	2.55	0.48	0.21
	3F		38.8	2,325	6.87	2.54	1.09	0.89	0.17
557	2R	1,500	23.1	1,390	6.42	1.68	1.09	0.58	0.72
	2F		22.8	1,370	1.63	3.89	0.26	0.47	0.42
	3F		37.4	2,245	1.11	0.67	0.89	0.37	0.50

*Essentially same speed condition as in Table I.

TABLE III

BUR	OPERATING CONDITION*	LOAD IN GRAMS	FUNDAMENTAL FREQUENCY	CALCULATED SPEED OF ROTATION	FUNDAMENTAL AMPLITUDE	AMPLITUDES OF HARMONICS			
						2	3	4	5
37	2R	200	20.2	1,220	4.50	1.91	2.45	0.76	0.76
	2F		19.9	1,195	26.60	2.11	3.02	1.22	1.07
37	2R	500	27.2	1,630	20.30	6.55	5.39	1.81	0.30
	2F		27.3	1,640	26.80	4.27	5.40	1.50	0.37
	3F		32.5	1,950	13.30	10.73	1.36	1.35	0.23
37	2R	1,000	22.4	1,345	6.89	13.20	7.24	1.20	0.93
	2F		30.0	1,800	9.71	3.19	0.73	0.50	0.86
	3F		46.4	2,780	20.50	4.23	2.72	1.23	0.24
37	2R	1,500	19.9	1,195	12.20	3.42	1.19	0.46	0.68
	3F		43.9	2,640	8.10	0.51	2.01	0.35	0.27
37	2R	2,000	17.2	1,030	28.00	1.71	3.41	0.99	0.58
	2F		21.7	1,300	4.64	1.66	1.86	0.69	0.73
	3F		36.0	2,160	2.96	5.60	3.07	0.50	0.39
39	2R	200	21.8	1,310	10.39	3.02	0.27	0.43	0.35
	2F		32.6	1,955	16.60	1.92	0.24	0.41	0.68
	3F		40.6	2,435	6.37	2.40	0.45	0.53	0.09
39	2F	500	34.6	2,075	19.20	1.33	1.27	0.68	0.33
	3F		43.6	2,620	7.43	3.36	0.92	0.69	0.38
39	2R	1,000	26.1	1,565	19.50	3.00	1.43	0.77	0.39
	2F		19.8	1,190	12.10	3.32	0.26	0.46	0.46
	3F		32.0	1,920	5.37	3.08	0.57	0.54	0.29
39	2R	1,500	17.4	1,045	14.84	0.61	1.54	0.65	0.67
	2F		16.5	990	3.35	1.61	0.68	1.90	0.60
	3F		37.5	2,250	4.45	2.27	0.80	0.24	0.62
39	2R	2,000	19.7	1,183	26.40	2.65	5.07	1.03	0.61
	2F		20.2	1,210	6.87	2.45	0.46	1.95	1.82

*Essentially same speed condition as in Table I.

and a pressure of ¼ lb. These two figures show graphically that the amplitude of the fundamental vibration is predominantly greater than the amplitude of the harmonic vibrations, and the relative value of each harmonic decreases as the number of the harmonic increases.

TABLE IV

BUR	OPERATING CONDITION*	LOAD IN GRAMS	FUNDAMENTAL FREQUENCY	CALCULATED SPEED OF ROTATION	FUNDAMENTAL AMPLITUDE	AMPLITUDES OF HARMONICS			
						2	3	4	5
2	2R	200	42.0	2,520	7.5	4.10	1.06	1.22	1.74
	2F		39.6	2,370	16.9	9.80	2.09	2.54	0.26
	3F		53.0	3,180	12.8	0.49	1.44	0.93	0.34
2	2R	500	49.4	2,965	18.8	2.01	0.88	0.04	0.25
	2F		41.6	2,490	15.0	3.80	0.22	0.18	0.19
2	2F	1,000	48.8	2,935	23.7	1.00	0.52	0.30	0.74
	3F		64.0	3,840	5.2	1.32	0.44	0.08	0.06
2	2R	1,500	38.5	2,310	17.0	5.20	0.70	0.40	0.43
	2F		49.5	2,970	14.8	1.76	0.40	0.06	0.03
	3F		47.9	2,870	3.78	1.14	0.18	0.04	0.10
2	2F	2,000	39.7	2,380	2.1	1.06	0.44	0.22	0.04
	3F		58.5	3,510	6.9	2.90	0.21	0.26	0.02
4	2R	200	51.9	3,120	25.5	0.42	0.32	0.13	0.13
	2F		40.2	2,410	13.6	0.90	0.89	0.19	0.27
	3F		47.8	—	12.7	1.01	0.22	0.19	0.08
4	2R	500	50.0	3,000	23.2	1.78	1.03	0.43	0.32
	2F		45.0	2,700	19.1	2.48	1.01	0.16	0.15
	3F			—	21.0	3.77	0.97	0.42	0.14
4	2R	1,000	63.0	—	12.8	2.47	0.55	2.39	0.20
	2F		47.6	2,850	8.45	0.88	1.87	0.73	0.18
	3F		61.0	3,660	19.5	5.27	2.15	1.56	0.78
4	2F	1,500	44.3	2,660	9.62	3.05	1.61	0.43	0.24
	3F		77.8	4,670	10.4	1.18	0.83	0.23	0.25
4	2F	2,000	49.2	2,940	7.5	1.57	0.58	0.16	0.05
	3F		82.4	4,940	12.7	0.81	0.19	0.13	0.07

*Essentially same speed condition as in Table I.

The curves in Fig. 4 show the relative amplitude of the fundamental vibration in relation to the pressure applied for the five burs studied. They show in general that, as the pressure is increased, the amplitude of the fundamental vibration decreases when the bur is driven in the forward direction and increases as the bur is driven in the reverse direction (lower half of Fig. 4). There appears to be some tendency for the fundamental vibration to increase as the bur size is increased, although more study is desirable to establish definitely such a relationship.

The relative amplitude of the third harmonic as a function of the pressure applied is shown in Fig. 5. In general, much the same conclusions can be drawn from these curves, with a few exceptions, as from those shown in Fig. 4, except that the changes are not so pronounced.

In Fig. 6 are represented the same data as in Fig. 4, but replotted to show the relative amplitude of the fundamental vibration as compared to the speed of

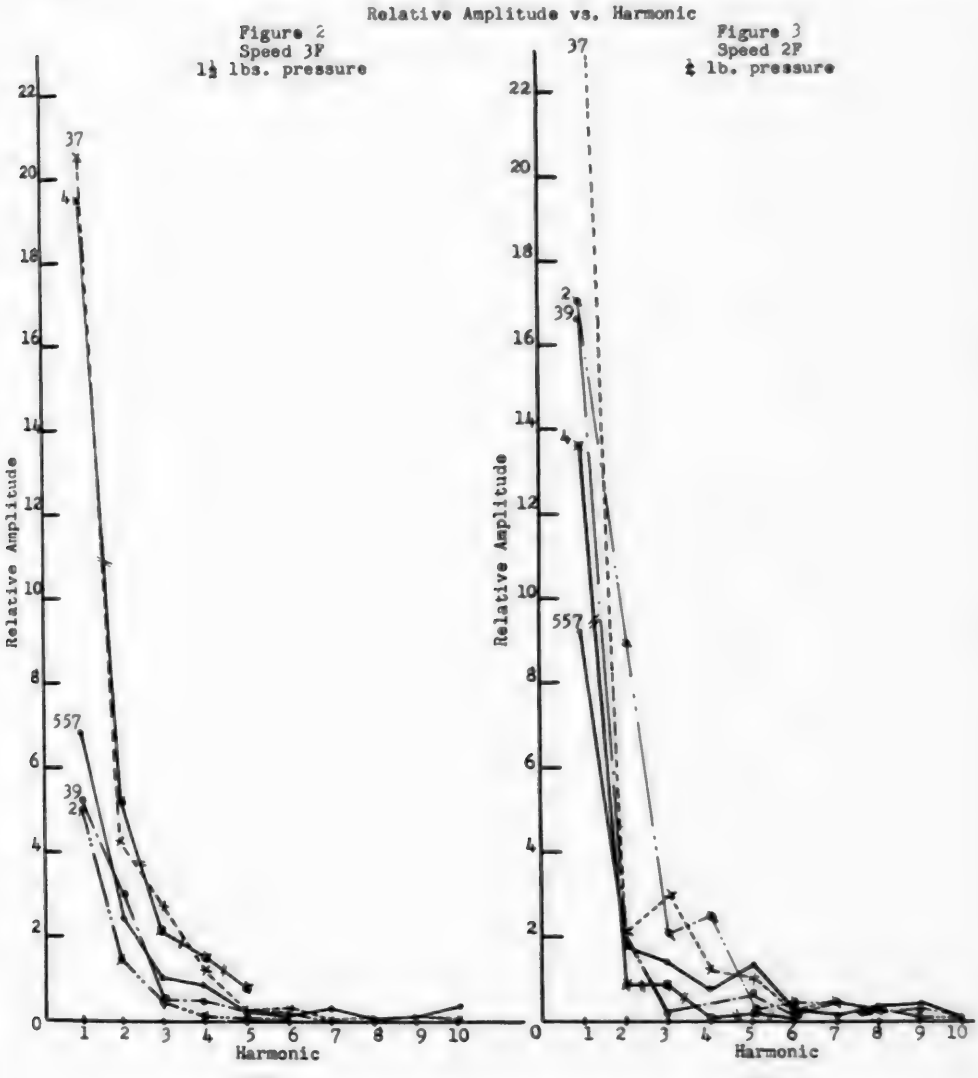

Fig. 2. Fig. 3.

Fig. 2.—Relative amplitude of various vibrations at Speed 3F and 1½ lbs. pressure.
Fig. 3.—Relative amplitude of various vibrations at Speed 2F and ¼ lb. pressure.

rotation at pressures of ¼ and ¾ lbs. The speed of rotation was calculated from the fundamental frequency of the vibration. When operating at these light pressures, a pronounced tendency is shown for the amplitude of vibration to decrease as the speed is increased. This prevails for both the forward and reverse directions of operation.

With the application of greater pressures of 2 and 3 lbs., as shown in Fig. 7, there is a less pronounced relationship between relative amplitude and speed

Figure 4
Relative Amplitude of the Fundamental vs. Pressure

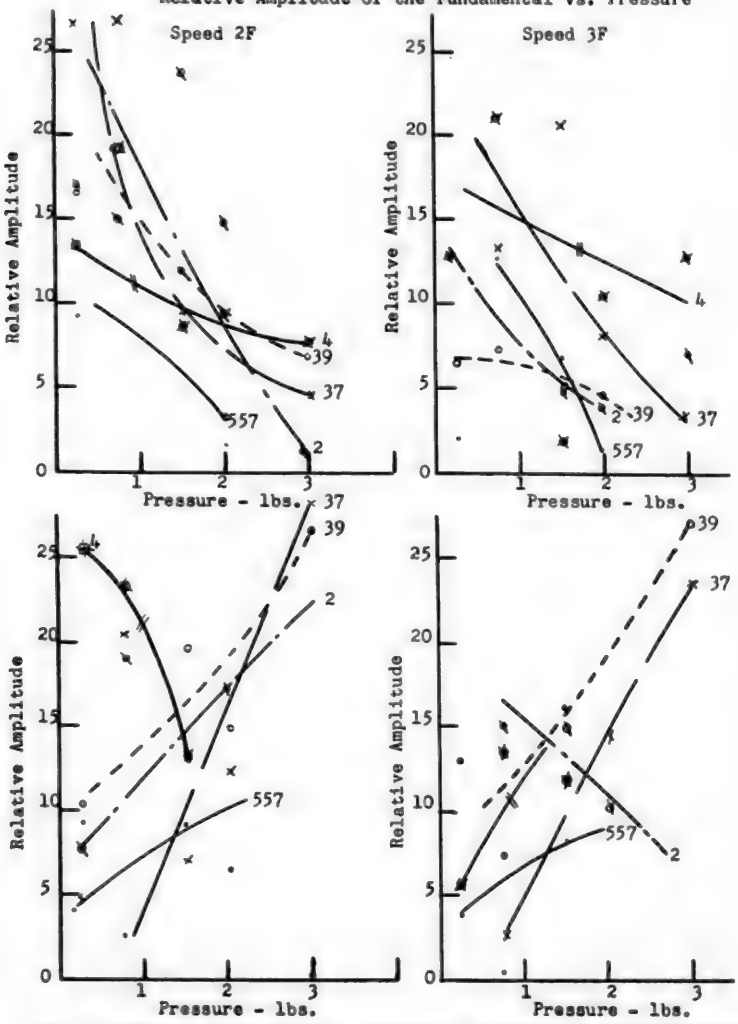

Fig. 4.—Relative amplitudes of fundamental vibrations at various pressures.

of operation. The slopes of the curves, however, are much more gradual at the higher pressures than at the lower pressures represented in Fig. 6. This characteristic, and the lack of agreement in trends at heavier pressures, may be due to several factors, one of which is perhaps the accumulation of chips in the cutting area.

A brief study has been made of the relative amplitude of the third harmonic developed at different speeds with light pressures of operation. These values are shown in Fig. 8. In general, there is a decrease in amplitude with an increase in speed for this third harmonic, which is similar to the trend shown by the fundamental vibration represented in Fig. 6. As pointed out previously,

Figure 5
Relative Amplitude of the 3rd Harmonic vs. Pressure

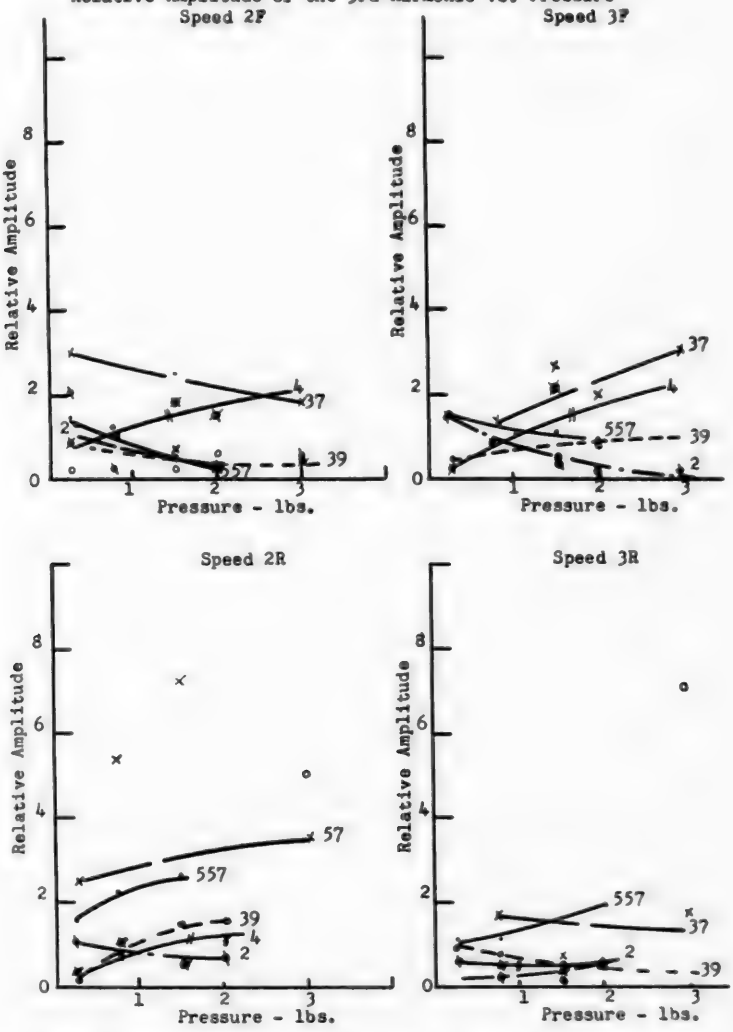

Fig. 5.—Relative amplitudes of third harmonic at different pressures.

however, the relative amplitude of this harmonic is considerably less than that of the fundamental vibration.

Photographs of oscillographic waves are shown in Fig. 9. These waves were produced at a speed of 2F and a load of 200 Gm. (¼ lb.) on the handpiece, when using a No. 557 bur, a No. 37 and No. 39 inverted cone bur, and a No. 2 and No. 4 round bur. A photograph of the vibration from an unloaded handpiece operated at the same speed is also included for comparison of the wave shape. No attempt should be made to compare the amplitudes of the wave in each photograph.

Figure 6
Relative Amplitude of the Fundamental vs. Speed of Rotation
¼ lb. pressure

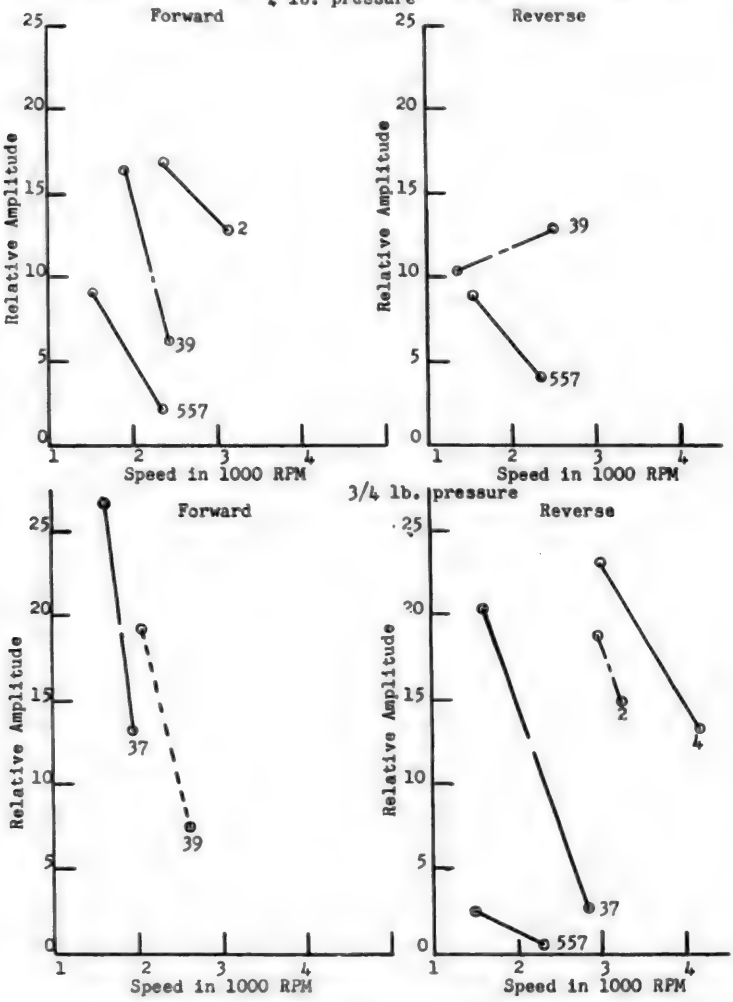

Fig. 6.—Influence of speed at light pressure on relative amplitude.

VIBRATIONS FROM THE AIR-DENT UNIT

Some study has been made of the vibration characteristics developed from the use of the gas-propelled abrasive to cut tooth tissue with the instrument known as the Air-dent Unit. Using the same method of detecting vibrations as described for the conventional handpiece, the stream of abrasive was directed from different angles toward the block of ivory being cut, on which the needle of the pick up was resting. With the abrasive directed toward the ivory block in such a direction as to be parallel with the plane of maximum sensitivity of the pick up, a relative amplitude of only 0.443, with a frequency of approximately

Figure 7
Relative Amplitude of the Fundamental vs. Speed of Rotation
2 lbs. pressure

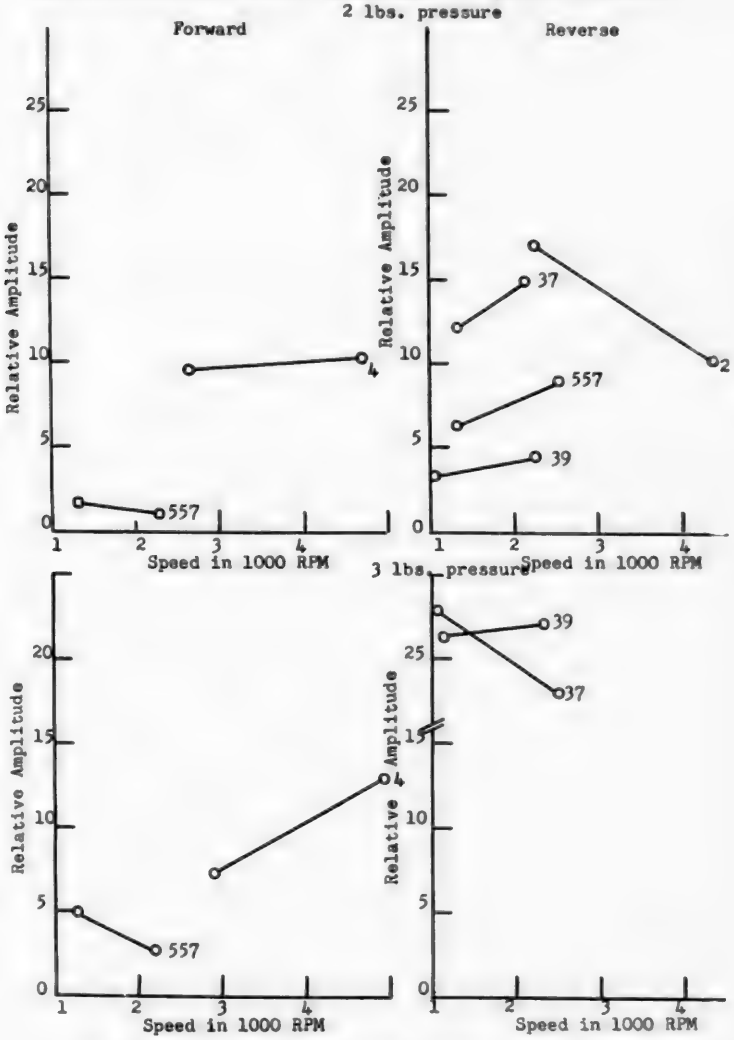

Fig. 7.—Influence of speed at heavy pressure on relative amplitude.

600 C.P.S. was obtained. As a possible source of vibrations during operation, or by comparison with more conventional rotary cutting instruments, it is not considered that this vibration is of practical significance.

DISCUSSION OF RESULTS

Throughout this study it has been evident that very little information is available to serve as a guide, or as a basis of comparison for values obtained on vibration characteristics. As the study progressed it became increasingly evident that many variable factors of operation must be well controlled if results

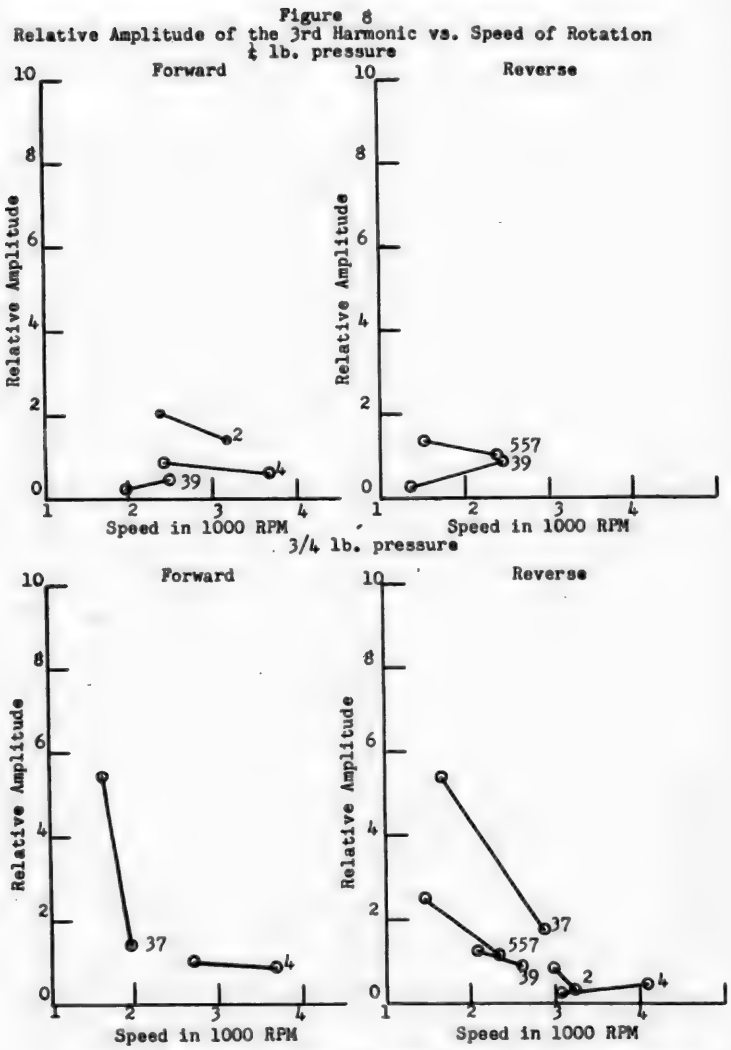

Fig. 8.—Influence of speed at light pressure on third harmonic.

of more than qualitative character are to be obtained. Since the study was initiated there have been several improvements and refinements introduced into the method of detection and measurement of the vibrations produced. It is believed that these alterations in method should make the continuation of this study less difficult in comparing other cutting instruments and conditions of operation.

In this initial report a comparison of only a few steel burs is described, when operated at conventional, relatively slow speeds, and at pressures which prevail at average operating conditions. From an examination of the data and

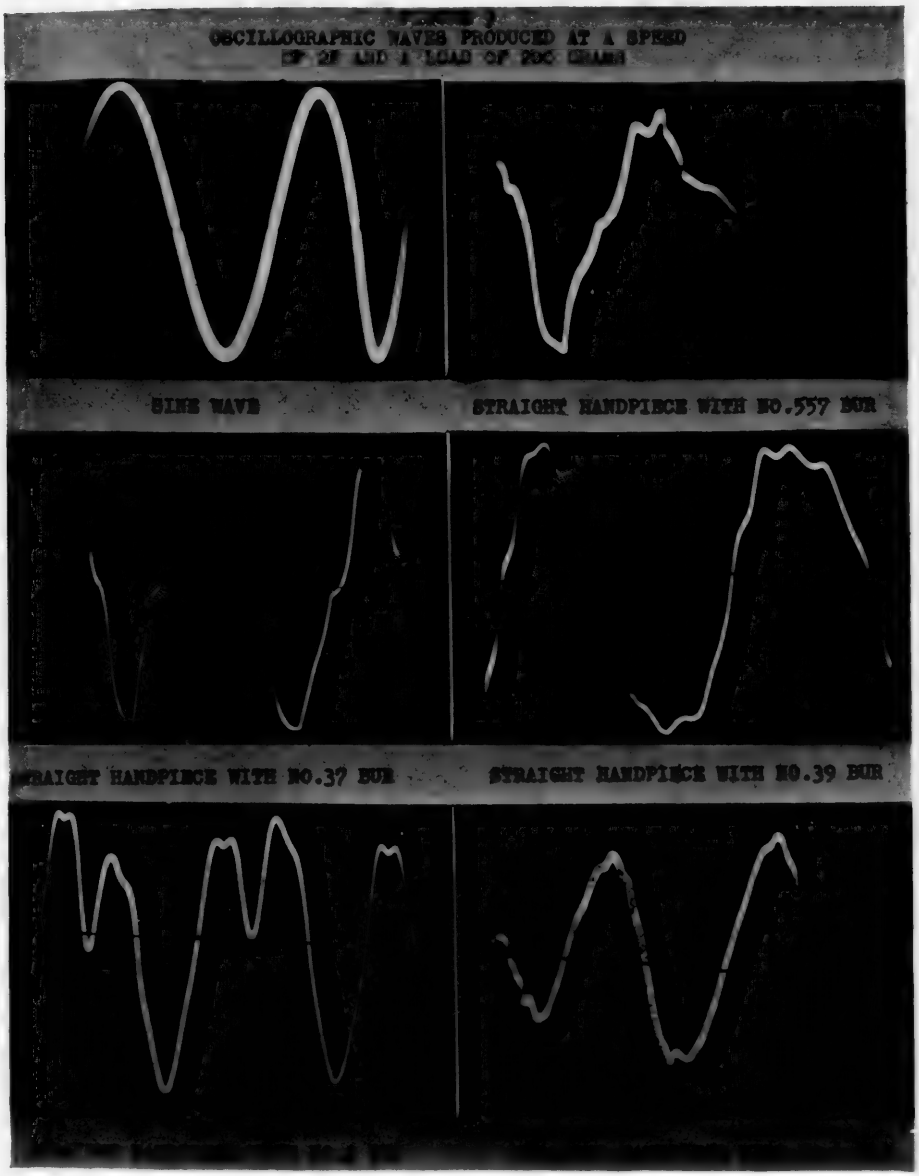
OSCILLOGRAPHIC WAVES PRODUCED AT A SPEED
SINE WAVE
STRAIGHT HANDPIECE WITH NO.557 BUR
STRAIGHT HANDPIECE WITH NO.37 BUR
STRAIGHT HANDPIECE WITH NO.39 BUR

Fig. 9.—Photographs of oscillograph waves with sine waves.

graphs presented there has been observed, in general, several characteristic trends which appear to exist. It is these trends which have been described in this report.

As the study of vibration characteristics of the handpiece has developed, it has become more evident that in order to understand fully the operating charac-

teristics of the rotating cutting instrument, the vibration should be correlated with not only the temperature change during cutting, but also with the efficiency of tissue removal of various instruments and conditions of operation. For example, it has been observed,[6] in the study of thermal changes developed during the cutting of tooth tissue, that nearly a linear relationship results in which the temperature change varies directly with either the pressure applied or speed employed. It would seem therefore that there are two compensating influences which exist during the cutting operation. Namely, as the pressure is increased, the vibration is decreased as observed in this study, but at the same time the temperature is increased. At light loads of operation, when the speed is increased the vibration is rapidly decreased, but as the speed is increased the temperature is increased. At heavier pressures, the vibration decrease is less pronounced as the speed is increased, but the rate of thermal change is nearly identical with that of light loads. Hence, from a practical standpoint both the temperature change and vibrations developed must be noted for each condition of operation. In addition, the practical aspect of the amount of tissue removed in any given period of time must be recognized. The instrument which removes the most tooth structure with the least vibration and temperature increase, yet has the longest life in continuous operation, should be the most nearly ideal.

SUMMARY

Since the results described in this paper represent a portion of the work done on the problem, and since the study is still actively in progress, this should be considered as a preliminary or progress report. The results described were obtained by using the conventional handpiece and rotating dental instruments to cut ivory. The data presented are considered to be applicable only for the test conditions described and, while they perhaps show a trend to be expected when operating on teeth in the mouth, studies will be made to verify the mouth conditions.

In this study certain characteristic trends were observed, and while some specific exceptions were also noted, the prevailing observations justify the establishment of the trends. Throughout the study the fundamental vibration wave has occurred at the speed of rotation of the handpiece shaft and changes with operating speed. The fundamental vibration wave, which is larger than any harmonic, is modified by different operating speeds and pressures, as well as by the choice of cutting instrument employed. In general, the amplitude of the fundamental vibration decreases as the pressure is increased when the bur is driven in the forward direction. When operating with light pressures there is the tendency for the amplitude of vibration to decrease as the speed is increased. This tendency prevails, but is less pronounced when operating at heavier pressures. In general, the slower speeds and lighter pressures have given the larger amplitude to the fundamental vibration wave, with gradually decreasing amplitudes for the second to fifth harmonics, beyond which the harmonic amplitudes are essentially the same to the tenth. While there is some tendency for larger bur sizes to show a greater vibration characteristic, more studies will be made of this relationship.

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MEASUREMENTS OF VERTICAL DIMENSION OF PROCESSED DENTURES

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MUCH is known concerning the horizontal linear change in dimension in the methyl methacrylate denture base materials. Many investigators have reported this phase of study.[1, 2, 3] The question has been raised as to what happens to the vertical dimension of the denture during processing and what part methyl methacrylate denture base plays in any change in vertical dimension. Some work has been done by Schuyler, Frederich, and Vaughan,[4] by making dentures and replacing them upon the articulator. Their conclusion was that they had obtained an "open-bite" by both the compression and injection method but no reason was given as to the cause of this "open bite." Atiyyah[5] employed a method that did not accurately measure the change in only the denture or in the denture base material, as some of the investing plaster had to be taken into the measuring scheme. Grunewald, Paffenbarger and Schoonover[6] have also investigated this subject. All of these investigations have left something to be desired with respect to knowledge of change in vertical dimension. It seemed that there should be some method by which this type of change might be accurately measured. If such a method were available, the numerous factors involved in this rather complex problem could be drawn into an enlightening picture. Consequently, the following testing apparatus and method were devised:

An all metal assembly machined to include a triangular base, 3 posts to support a metal horseshoe of 0.0617 inch thickness (the approximate thickness of one sheet of baseplate wax), and three tapered caps to represent teeth. The horseshoe was designed to fit into the posts, and the taper caps to fit over the pins in the horseshoe, making continuous metal to metal contact (Fig. 1). Each piece was numbered so that the assembly would always be fitted together in only one way.

Cap 1 was located back of the first incisors on the median line, Cap 2 was placed on left side in the position of third molar on the ridge, and Cap 3 on the right in place of the third molar. The distance from the top of the taper caps to the bottom of the metal triangle measured approximately one inch in height.

An elastomeric (V-B-M) mold was used as a form for making the stone cast. The metal horseshoe, triangle, and posts were fitted into the mold and a dental stone was poured around the metal form. The mix of dental stone was 30 ml. water to 100 Gm. of stone in all cases. When the stone was set, the metal and stone cast were removed from mold.

The taper caps were put in place, and wax (one thickness of baseplate wax, except upon the ridges where two thicknesses were used) was added to form

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an average baseplate, with the taper caps partially embedded in wax. (Fig. 2.) Measurement was made on the waxed-up case on all three caps, that is, the distance from the top of the taper cap to bottom of metal triangle was measured with a Brown & Sharpe micrometer which was always checked with a standard immediately before and after all recordings.

Fig. 1.

Fig. 2.

The entire assembly was then invested in a three section dental stone (Castone) mold. The second pour of investment was brought up to the tips of the taper pins so that measurement could be made after processing, without disturbing the investment. After the investment had set for at least one hour, the wax was removed. At this time, the metal horseshoe was also removed, creating a measured space to be packed with methyl methacrylate. Vernonite powder and liquid pink, used throughout this study, was mixed and packed according to the directions, with the addition of one extra trial closure, but omitting Cellophane. All excess was trimmed on each trial pack. The cast was painted with an alginate separator and closed. Most of the processing was done at

160° F. for 9 hours. After processing and bench cooling, measurements were made on the partially de-flasked case, and results recorded.

The average result of 8 cases is shown here, indicating an increase in vertical dimension. A minimum of flash on the lands of plaster was maintained; however, the line indicating the split mold remained. It is felt that these measurements are reliable to 5 ten-thousandths (0.0005 inch) of an inch.

INCREASE IN VERTICAL DIMENSION OF PROCESSED BASEPLATES

CAP 1 (INCHES)	CAP 2 (INCHES)	CAP 3 (INCHES)
0.006 ± 0.002	0.006 ± 0.002	0.006 ± 0.002

To obtain further information, the same setup was used except that the shape factor of the denture was eliminated (Fig. 3). In some instances, the taper caps were left in place, and in other tests, the taper caps were waxed high so that they could be removed when the wax was eliminated. When the metal caps were replaced by Vernonite, these taper caps were very carefully measured, and included in the computations. The space being replaced by resin was then the thickness of the metal horseshoe, plus the height of the metal taper cap.

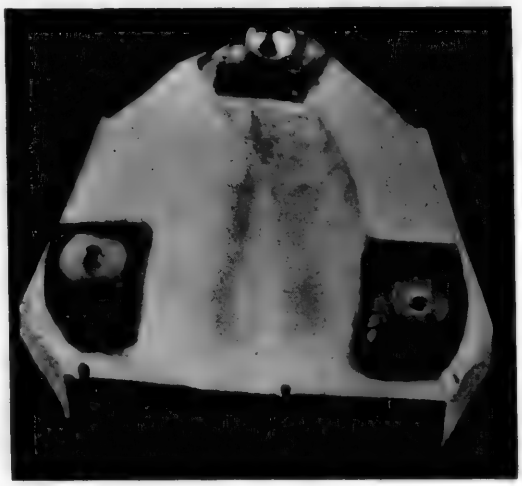

Fig. 3.

Results of these tests gave information on methyl methacrylate resin having no metal insert, nor any metal part which was held fast in stone, making it possible for the resin to contract without any restraining force to restrict its behavior. The vertical change in methyl methacrylate, as a change in material, resulted in a shrinkage of 0.40 per cent. This correlates closely with the often reported values for horizontal linear shrinkage. It is suggested that this figure may vary as the curing temperature is increased, or as the cooling rate is changed.

Where the metal cap was retained and the shape of the denture eliminated, the change in vertical dimension was of the order of +0.002 inch, indicating that

the shape of the denture may cause some change. At this point, a baseplate was sectioned across the ridge and down to the palatal area, and a photomicrograph was taken, showing the space on the lingual ridge side and down into the palate.

The next problem involved in this investigation was: "What effect on vertical dimension do artificial teeth have in a test of this kind?" Acrylic premolars were prepared and used in place of the taper caps, with all other factors being kept the same. Monel metal taper pins were cemented into and through the center of the premolars so as to continue to maintain metal to metal contact for purposes of original measurement. In one set of tests, the premolar was waxed up to the occlusal surface, eliminating all undercuts; in the other set of tests, the premolar was waxed low on the tooth, as it would be on a practical case, thus exposing the shape factor (undercuts, etc.), to the lock of the surrounding investing stone (Fig. 4). These tests were made on a baseplate, involving the shape factor of the denture as well as on "flats" where the denture shape was eliminated. Results were as follows:

CHANGE IN VERTICAL DIMENSION

BASEPLATES		FLAT PLATES	
Premolar undercuts eliminated (Inches)	Premolar held in stone (Inches)	Premolar undercuts eliminated (Inches)	Premolar held in stone (Inches)
+0.006	+0.014	−0.001	+0.001

Once again the shape of the denture seemed to play a definite part in increasing the vertical dimension. Also, the shape of the tooth, depending upon how firmly that tooth was locked in the stone, was a factor involved in vertical change. This may be why spot grinding is necessary on new dentures.

Fig. 4.

Since the taper cap setup correlated closely with figures obtained by using the acrylic teeth, the entire metal assembly was waxed up, measured, and sent to five different reputable laboratories for processing in their accustomed manner. The cases were then returned to our laboratory without the investment having been removed. The results of these processings are given in Table I.

Since it is very difficult to have either methyl methacrylate or vinyl-acrylic resin processed with practically no flash, most of the dentures processed will

retain a slight fin, which will increase the vertical dimension by a few thousandths of an inch.

In order to see if any difference would be found between injection molding and the conventional compression molding, a series of cases were made with each type of packing.

TABLE I

COMPARISON OF CHANGE IN VERTICAL DIMENSION OF BASEPLATES

MATERIAL	PLACE OF PROCESSING	CAP 1 (INCHES)	CAP 2 (INCHES)	CAP 3 (INCHES)	AVERAGE (INCHES)	FLASH
Vernonite P & L	V-B Research	0.006 ± 0.002	0.006 ± 0.002	0.006 ± 0.002	0.006	Minimum flash
Vernonite P & L	V-B Laboratory	0.016	0.012	0.017	0.015	Flash
Vernonite P & L	Select Dental Lab. 1	0.017	0.022	0.021	0.020	Flash
Vernonite P & L	Select Dental Lab. 2	0.005	0.006	Distorted Cap	0.006	Thin flash
Vernonite P & L	Select Dental Lab. 3	0.008	0.011	0.013	0.011	Flash
Vinyl-acrylic	Select Dental Lab. 4	0.015	0.010	0.009	0.011	Flash
Vinyl-acrylic	Select Dental Lab. 5	0.004	0.007	0.011	0.007	Flash

The Pryor and vinyl-acrylic types of equipment were used for the injection methods. The results are shown in Table II. It was concluded that no greater precision in vertical dimension was obtained by either the compression or injection methods on this series of tests.

TABLE II

COMPARISON OF CHANGE IN VERTICAL DIMENSION OF INJECTION AND COMPRESSION MOLDED UPPER DENTURE FORM

MATERIAL	METHOD	CAP 1 (INCHES)	CAP 2 (INCHES)	CAP 3 (INCHES)	AVERAGE (INCHES)	FLASH
Vinyl-acrylic	Injection	0.015	0.010	0.009	0.011	Flash
Vinyl-acrylic	Injection	0.004	0.007	0.011	0.007	Minimum flash
Methyl methacrylate	Pryor	0.009	0.011	0.011	0.010	Flash
Methyl methacrylate	Pryor	0.014	0.013	0.013	0.013	Flash
Vernonite P & L	Compression	0.006 ± 0.002	0.006 ± 0.002	0.006 ± 0.002	0.006	Minimum flash

Another series of tests were made to ascertain the effect of excessive pressure during the packing of the methacrylate resin. This excessive pressure is created by a rapid closure of an overfilled denture mould, reducing the time for escape of excess resin from the mould cavity. Would this additional pressure result in an increase in the vertical dimension of the finished denture? Would one type of investment material counter additional pressure more effectively than others?

The same testing device, using the metal horseshoe and metal taper caps was employed. The types of investments used were dental plaster, dental stone (Castone), and a still stronger dental stone (Duroc). The tests on each material

were made in duplicate. The results are shown on Table III. It can be concluded from this series of tests that excessive pressure during packing of the denture can alter vertical dimension of the prosthesis.

TABLE III

THE EFFECT OF EXCESS PRESSURE IN PACKING ON VERTICAL DIMENSION OF METHACRYLATE DENTURES

INVESTMENT	POSITION OF TAPER PINS			
	NO. 1 (INCHES)	NO. 2 (INCHES)	NO. 3 (INCHES)	AVERAGE (INCHES)
Plaster	0.014	0.017	0.015	0.015
Stone (Castone)	0.011	0.012	0.012	0.012
Duroc	0.009	0.009	0.007	0.008
Castone (No excess pressure)	0.006 ± 0.002	0.006 ± 0.002	0.006 ± 0.002	0.006

SUMMARY AND CONCLUSIONS

1. Methyl methacrylate resin when cured at 160° F. for 9 hours contracted 0.40 per cent in the vertical dimension.

2. The shape factor of teeth (undercuts, etc.) will add, in varying degree, to the vertical dimension of a denture (ridge to cusp tip), depending upon how firmly the teeth are locked into the investing medium.

3. The shape factor of the denture plus the horizontal linear contraction tends to raise the denture from the cast in the palatal area and over the lingual ridge areas to a very minute degree, adding somewhat to the vertical dimension ridge to cusp.

4. Any fin or flash on the denture will add to the vertical dimension.

5. The control of vertical dimension was equally good for injection and compression technics of processing dentures.

6. Excessive pressure during packing of the denture may increase the vertical dimension of the denture. The change in vertical dimension may vary with the type of investment used.

7. A testing device and technic for measuring vertical dimensional changes was developed which can be used to study this property for other materials and denture technics to a high degree of accuracy.

The author wishes to thank Mr. H. M. Vernon for his idea for the testing of change in vertical dimension as well as his machining of the testing device and Dr. J. A. Malcolm for his fine cooperation on this problem.

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PERMEABLE STRUCTURES IN NORMAL ENAMEL

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INTRODUCTION

THE PROBLEM of the permeability of dental enamel has been studied by every serviceable method by numerous investigators and, as the outcome, a great amount of very heterogeneous material is at our disposal. On analyzing this material it appears that the investigations differ not only as to the methods applied and the materials examined, but even as to the objectives. One cannot fail to get the impression that many authors made their investigations without duly realizing how their results were to fit into the pattern of enamel research as a whole.

First of all, we have tried, by properly arranging the data from the available literature, to form some idea of the various aspects the problem of permeability presents. The subject has been considered from the following points of view:

1. *Objectives*

A. One may engage in the problem of permeability for the exclusive purpose of studying possible physiological processes in the enamel cap as a whole.

B. One may attempt to ascertain which microanatomical parts, if any, are permeable and which are not.

In the former case the investigations will be chiefly of a physiological nature, in the latter they will be mainly morphological. In accordance with this, methods as well as results are bound to differ. So long as the synthesis between the physiology and morphology of enamel is so regrettably far away, it will be necessary to distinguish between these two extreme sides of the problem in order to avoid inferring too readily the existence or nonexistence of physiological processes from purely morphological data. In our field this mistake is made if, for instance, from otherwise faultless experiments with dyestuffs one endeavors to prove or to disprove the vitality of enamel.

2. *Methods*

The methods adopted until now may be brought under these heads:

A. Permeability tests with substances to be identified chemically.

B. Permeability tests with substances to be identified physically.

I. By means of radioactive isotopes.

II. By means of dyestuffs.

With a view to ascertaining the permeability of the enamel cap as a whole (see point 1A) any of the three methods mentioned may be adopted, for morphological investigations only method BII has proved practicable.

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3. *Material*

We should first of all distinguish between

A. Experiments in vitro.

B. Experiments in vivo.

Further, it may be of importance whether the experiments are made on lower animals or on human beings and which teeth are chosen for the purpose (dentition, type, age, and vitality of the pulp).

Last, when judging of the results of experiments in vivo one should take into account any changes (through capping, drilling, or grinding) which may have been brought about in the tooth in question or its environment.*

We suggest that in the present stage of our knowledge of the permeability problem the following questions should first of all be further investigated:

1. Does any displacement of matter take place in the enamel and if so, what substances are displaced, what is their fate, and what is the driving force in their displacement?

2. Are permeable structures demonstrable in unimpaired enamel in a normal mouth?

The first questions are clearly of the "physiological" type, whereas the latter seems to be purely morphological in character. Morphology is only part of it, however, for it is emphatically stated that the enamel should be "unimpaired in a normal mouth." Apparently it is as yet uncertain whether the enamel of an extracted tooth behaves in the same way as "unimpaired enamel." It is the physiological part of the second question, introduced by this uncertainty, which calls for the preservation of "physiological conditions" during the experiments designed to solve this question.

Accordingly, on analyzing the second question, which must have been formulated in a similar way by most investigators, we have been struck by the fact that although their conclusions furnish the answer to it, their methods do not always pay due regard to the requirements which this question implies. Thus, to give an instance, it is most remarkable how rashly some authors, on the ground of experiments in vitro, have jumped at conclusions as to what will happen in a normal mouth (e.g., Atkinson,[2] Bouyssou,[13] Hartingsvelt,[21] Herzig,[22] and Smreker[35]).

Others have duly realized the necessity of experiments in vivo, but even so they have not always sufficiently reckoned with the preservation of the physiological conditions of the teeth experimented upon. Again, it must not be assumed, a priori, that the enamel of teeth subjected to drilling will behave in the same way as that of intact teeth.

As we proceeded, it became evident to us, that it is impossible to answer both the above-mentioned questions at the same time from the results of one experimental method. For each one of the two questions requires appropriate methods, which at the time cannot be applied to attempts at answering the other one.

*In order to obviate the need of giving a confused summary of the investigations made by the authors consulted we have restricted ourselves to indicating, in the references, where appropriate, the contents of their communications with the help of the letters and figures used above (e.g., a morphological investigation carried out with dyes in vivo is designated: 1B-2BII-3B).

The presumed permeable structures in the enamel cannot be recognized as such until after they have been made visible in the microscope with the help of dyes. Dyes, suitable for this purpose, are not found in the entirely normal mouth. Accordingly, we are compelled to study the microscopic pathways in the enamel after application of dyes which are substances foreign to the body. This precludes any attempt at solving, with the help of dyes, the first questions (the substances displaced, their fate etc.). Radioactive isotopes on the other hand, being especially well suited to problems of a purely physiological nature, are of less value in the study of the pathways, for in a microscopical section they cannot be localized as accurately as dyes (Bodecker[11]).

In view of the above considerations we now are in a position to suggest a restricted but practicable investigation, intended to answer the second question only: What are the pathways of a dye penetrating into normal enamel? To our knowledge only Wannenmacher,[44] Lefkowitz and Bodecker,[27] and Kanner[25] made attempts at dyeing enamel from the outside under normal or nearly normal conditions. As their results do not agree very well we felt the necessity of repeating their experiments with a more appropriate technic.

EXPERIMENTAL

Dyes may be made to permeate the enamel (a) from the outside (application to the surface of the enamel) and (b) from within (e.g., by injection into the bloodstream). We chose the first method and applied it to dogs, many of which were very young, so that we were in a position to include deciduous teeth in our experiments. It is stated in the literature, in connection with other experiments, that their enamel is readily permeable (Fish,[16] Bodecker,[10, 11] and Volker[43]).

The application of the dyes was effected in two different ways:

1. By means of contact fillings. This method was first applied to older dogs.* In some of the permanent incisors ample cavities were drilled and filled with the dye in powder form. These cavities were then sealed with oxyphosphate or silicate cement (covered with varnish) in such a way that over a considerable area only a very small space remained between the filling and the unimpaired adjacent tooth. During the operation this adjacent tooth was left untouched by the bur. We knew from earlier experiments that dyes diffused through and along cement fillings sufficiently to ensure a continuous supply to the saliva bathing the contiguous tooth. The dogs survived this treatment by 1 to 4 days. (For an ingenious method in this connection see also Bodecker and Lefkowitz.[9])

2. When it became apparent, from method 1, that even after an application of short duration coloring of an unimpaired tooth occurred, the following experiment was devised: On the deciduous canines of pups, anesthetized by Pentothal Sodium, wads of cotton soaked in a solution of dyestuff of a known strength were applied. The advantage of this method was that, besides the strength of the solution of the dye applied being known, the duration of the

*We had these animals placed at our disposal by the courtesy of the director of the Physiological Department of the University of Utrecht.

application to each individual canine could be ascertained accurately. These periods varied from 2 to 30 minutes. In each case, in order to check results, one of the canine teeth was extracted before the experiment was started. It was further treated in exactly the same way as the colored ones.

Dyes.—The dye used should first of all have no known toxic or astringent action, for otherwise the preservation of the physiological circumstances of the tooth would be endangered by the mere application of the dye. Accordingly, the use of such substances as silver nitrate cannot be considered.

During preliminary experiments methylene blue had been used, but it appeared that accurate localization of small quantities of this dye was impossible in thin sections because, microscopically, the difference between the blue tint of the dye and the dark diffraction lines of the prism sheaths is too slight (see also Urbantschitsch[39] and Gustafson[19]).

This difficulty was met (in accordance with a suggestion by the director of this department, Prof. Berkelbach van der Sprenkel) by using fluorescent dyes and filtered ultraviolet light (see also Wannenmacher[44]), for which purpose we had to choose a dye fluorescing in a tint different from that of the enamel. An additional advantage of some fluorescent dyes is that in ultraviolet light they are discernible in far greater dilution than most dyes in white light.

It appeared that, even when greatly diluted, Trypaflavin will show a clearly visible yellowish-green fluorescence which stands out distinctly against the bluish fluorescence of the enamel. Therefore, whenever in the course of this discussion structures are referred to as permeable this means that Trypaflavin was demonstrable in these structures.

Microscopic Technic.—After the application of the dyestuff the teeth were extracted. From that moment it was attempted to prevent any further displacement of the permeated dye. With this object in view the following technic was adopted: The pieces were quickly frozen in liquid aid by the Altmann-Gersh[17] freezing-drying method to prevent any diffusion. Because of the very fast drop in temperature the water in the object will freeze into such tiny crystals that no microscopically discernible deformation is to be feared. Subsequently, the block of tissue is quickly transferred to a vessel in which a temperature of approximately –33° C. is maintained and which communicates with another vessel containing P_2O_5. In these vessels a vacuum is maintained. The ice will sublime into water vapor, which will eventually be absorbed by the highly hygroscopic P_2O_5. The diffusion of the water molecules from the pores of the object to the P_2O_5 will proceed far more quickly in vacuo. The tissue is kept under these conditions for a fortnight. In order to prevent a displacement of the dye in the dried enamel during the process of sawing and grinding the pieces of tissue were subsequently infiltrated with liquid monomer of methyl methacrylate. After a fortnight the monomer was replaced by a treacly prepolymerized form of the plastic while after another fortnight the final polymerization was effected (Puckett[33]).

In order that the freezing-drying process and the inhibition with methyl methacrylate may be effected as thoroughly as possible, it is desirable that the

teeth be divided into small pieces. This is achieved without our intervention through cooling in liquid air. By a method described by Jansen,* sections 0.080 mm. thick were sawed and then ground down until they were no more than 0.005 to 0.020 mm. thick.

Fluorescence Microscope.—The source of ultraviolet light used consisted of a water-cooled super high-pressure mercury arc lamp, Philips S. P. 500. The light from this lamp was directed parallel by a system of quartz lenses, and both the visible light and any radiation of still greater wave-length were eliminated by a combination of filters consisting of a water-cooled $CuSo_4$-curvette (5 cm.-long) and a nickel-oxide glass.

The microscope was provided with a surface-coated aluminium mirror and a quartz condenser. Nonfluorescing object slides were used and "Euphos" cover slips, which prevented ultraviolet light rays from falling into the objective of the microscope. Paraffin oil was used as a medium for observation.

RESULTS

A. *Undyed Teeth.*—In sections from uncolored teeth, when treated according to the method adopted by us, enamel shows a much weaker fluorescence than dentin, predentin, and pulp. Enamel fluoresced a pale blue whereas dentin, even in thin layers, shows a bright sky-blue fluorescence. The fluorescence of predentin and pulp, and generally that of the granular layer in the dentin as well, was bluish green.†

It was as a rule impossible to discern in the enamel any of the details which are so characteristic of sections from colored teeth under high power. In one case we were able to observe the pattern of the prism in an unstained tooth, but in this case it was the prism bodies, that fluoresced in a grayish hue, whereas the interprismatic substance and the prism sheaths remained dark.

B. *Dyed Teeth.*—It made no essential difference whether the dogs had been treated according to the first or the second method. In almost all cases the dye proved to have penetrated into both the enamel and the dentin, even in those cases in which the dye had been applied (to deciduous teeth) for only a few minutes.

In a few instances in deciduous as well as in permanent teeth, the coloring of the dentin was more marked than that of the enamel on top of it. We had already found this to be the case in experiments with methylene blue in vitro. In none of these instances were we able to establish any damage to, or any abnormality in, the enamel which might account for this phenomenon. We observed in the case of a tooth which had been very slightly damaged locally by the bur during the preparation of the adjoining tooth that damage to the enamel surface will result in an increased penetration of the dye. In the spot affected, the dye had penetrated with distinctly more telling effect. Whether

*Jansen, M. T.: An Improved Method for the Preparation of "Serial" Sections of Undecalcified Dental Tissues, *J. D. Res.* **29**: 401, 1950.

†In one dog we noticed in the enamel of the tooth that had not been treated with Trypaflavin, a brownish-yellow fluorescence, which seemed to be distributed in a way similar to that of the dye in colored teeth. It is obvious that the sections from the colored teeth of this particular dog were not used in this investigation, for the yellowish-green fluorescence of Trypaflavin could not be distinguished from this abnormal fluorescence with sufficient certainty.

this enhanced coloring is to be attributed to increased permeability on account of damage or to local rentention of dyestuff we cannot tell.

The subsequent description of the microscopic pictures refers to the distribution of the dye in the enamel, further penetration into the deeper tissues being mentioned incidentally only. As a matter of fact, when Trypaflavin is used, our observations both in dentin and pulp are less positive because of the color and intensity of the specific fluorescence of these tissues.

In general, the dye was not found to have been distributed evenly through the enamel body; in cross sections three layers could be distinguished. In most cases there was a more distinctly colored central zone separated from the dentino-enamel junction by a zone which had remained practically without dye. The outer zone, too, contained less coloring matter, but the line of demarcation between it and the central zone was much less clearly marked than that between the central zone and the zone bordering the dentin. Moreover, we often found the outermost layer of the outer zone as if it were drenched with dye (Fig. 1). The central zone coincided more or less with the region in which the Hunter-Schreger bands are normally most clearly marked.*

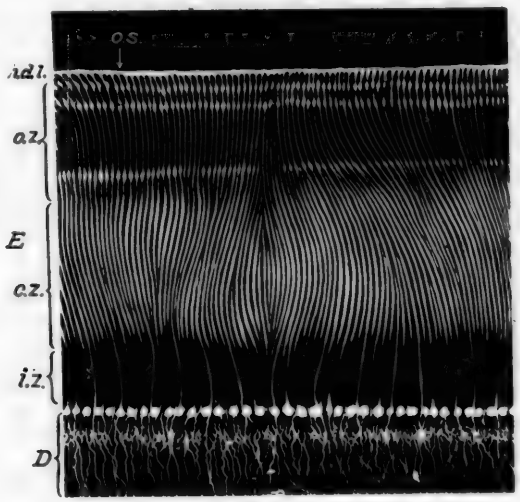

Fig. 1.—Transverse section of enamel (*E*) and dentin (*D*) of a dog's permanent canine. Dyed for two days.

O.S.: outer surface of enamel; *h.d.l.*: homogeneously dyed outer layer of enamel; *c.z.* central dyed zone of enamel; *o.z.* and *i.z.*: less intensely dyed outer and inner zone.

The prism sheaths of adjacent prisms have been represented by one single line only. Note Retzius' lines in outer zone. The row of white spots in the outermost layer of the dentin represents the granular layer.

Apart from the zones mentioned, accumulations of dyestuff are found in Retzius' lines which run more or less parallel to the surface of the enamel in cross sections through the teeth. These linear accumulations, however, are much narrower than the zones already mentioned and they occur far less regularly (Fig. 1).

*We doubt whether the enhanced coloring of this region can be explained in the way Staz[37] suggests for his stained semidecalcified sections. The great differences in the strength of coloring leads us to believe that the permeable structures in this region do really contain more dyestuff than elsewhere.

At right angles to these are the lamellae which even at low magnification we perceived running through the enamel in bright yellowish-green lines (cracks, which may occur during the sawing and grinding processes, will not fluoresce). On either side of the lamellae we sometimes noticed a narrow zone in which the enamel contained less dye through the entire thickness than elsewhere. Occasionally, ramifications sprang from the lamellae, which faded away into the enamel. It struck us that in all the zones before mentioned the lamellae had been colored with equal brightness. At the points where they reached the dentino-enamel junction a local accumulation of the dyestuff in the dentin was noticeable.

While studying the sections it appears that clearer pictures are obtained as the sections are thinner. Sections from heavily dyed teeth can be observed with greater advantage with an oil-immersion lens in places where the enamel is only one prism layer thick; in more faintly dyed sections of this thickness, so little is seen that one has to have recourse to layers of greater thickness. Under high power we found the following:

1. In most cases, even after prolonged application, the dye proved to be localized exclusively in the prism sheaths. In these cases we noticed, between two undyed prisms, both in the longitudinal and in the cross sections, the two green lines of the two prism sheaths enclosing the undyed interprismatic substances* (Fig. 2). The colored prism sheaths could be seen particularly clearly in the central zone but occasionally also, in the region next to the brightly colored outer layer of the outer zone and in the spreading branch of a lamella.

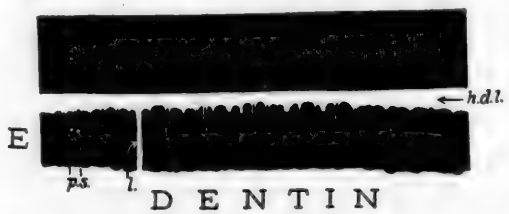

Fig. 2.—Very thin transverse section of deciduous canine of a dog, near cervical margin of the enamel. Dyed for 5 minutes.

E: enamel; *o.s.*: outer surface of enamel; *h.d.l.*: homogeneously dyed layer; *p.s.*: prism sheaths of two adjacent prisms, enclosing undyed interprismatic substance; *l*: lamella.

2. The homogeneously dyed most superficial layer of the outer enamel zone proved, even under greater magnification, to allow of no discrimination between prisms, prism sheaths, and interprismatic substance. That this layer was really a part of the enamel could easily be ascertained by intermittent use of white and ultraviolet light. We must therefore assume that in this superficial layer the enamel is dyed uniformly.

3. In the bottom zone of the enamel, i.e., the zone bordering on the dentin, the dye was mainly visible not only in the lamellae but also in other lines at right angles to the amelodentinal border. The lines are distinguished from the prism sheaths (a) by not being observed in double lines; (b) by being on

*This was the usual picture. In the specimens from one dog, however, we found that the bodies of the prisms were dyed as well, at least locally. This was perhaps a case of local hypoplasia.

the whole dyed more brightly (as if they were tiny lamellae) and (c) by being situated at far greater distances from each other than was compatible with the width of the prisms (Fig. 1).

Because of the appearance of these lines in white light we feel inclined to look upon them as parts of tufts which, however, are not plainly distinguishable in the case of dogs. Sometimes there was coherence of the green fluorescent dentin tubules penetrating into the enamel with the green tinted lines and sometimes there was not.

4. As far as we could make out, the colored Retzius' lines were formed of rows of thickened parts of the green prism sheaths* (Fig. 1).

The images described mainly were observed after periods of application of from 15 to 20 minutes. As has been stated, no essentially different images were seen in the case of dogs treated according to method 1, although in the latter the coloring of the lamellae and their ramifications predominated on account of the slighter coloring of the rest of the enamel.

In the case of very short applications the pictures were mainly the same. Even after an application of a ½ per cent Trypaflavin solution during 2 minutes, clearly discernible dyeing of deciduous teeth was found. Apparently the dye is able to penetrate through the whole thickness of the enamel layer of these teeth within this short period.

DISCUSSION

Those structures which in our experiments were dyed with Trypaflavin are described as permeable. One should remember, however, that there may be several permeable structures in the enamel which had not been reached by the Trypaflavin molecules or from which the dye, in spite of the precautions mentioned, was lost. Provided that these restrictions be borne in mind we suggest that the following permeable structures are demonstrable in normal enamel of the dog, dyed from without: prism sheaths, lamallae, and tufts.

Analogous experiments were made by Fish.[16] This investigator attained similar results with pups, but he made use of a cap shutting the teeth off hermetically, so that conditions cannot be considered normal. Moreover, Fish assumed permeability only, in the enamel of pups, whereas we have established permeable structures in older specimens as well.

Kanner[25] experimented on human beings, using proper precautions for maintaining normal conditions. He observed permeation of the enamel by methylene blue but unfortunately failed to refer to any pathways. He emphatically stated that dye was absent in the dentin, and he therefore assumed the amelodentinal border to be a membrane (see also Wannenmacher[44] under "Experiments with $AgNO_3$ in vitro"). This conception has not been corroborated by our experiments.

Wannenmacher[44] and Berggren[5] made use of fluorescein and methylene blue. They applied their dyes properly, but failed to establish any penetration in the case of human teeth. This also applies to Lefkowitz and Bodecker,[27]

*We have not been able to find a trace of any bayonet-like deflection of the prism bodies (Gustafson[20]).

in their experiments with $KMnO_4$ on dogs' teeth. They even went as far as to state they had proved that dyes cannot penetrate into normal enamel from outside.

Our findings do not indicate the existence of permeable structures in vivo that had not been demonstrated previously in vitro by a multitude of authors. Thus our microscopic investigations yielded, paradoxically, new physiological rather than morphological data: the pathways, already described extensively in extracted or otherwise damaged teeth, really can act as such with regard to Trypaflavin in teeth under normal conditions.

Our method did not enable us to come to any further conclusions as to the physiology of the enamel. As Trypaflavin does not occur in a normal mouth our microscopic images cannot tell us whether any displacement of substances really does take place along the pathways under normal conditions. At most it may now be assumed that substances which behave physicochemically in a way similar to Trypaflavin, and consist of molecules of equal or smaller size will permeate the enamel. We are not able to state what substances may fall under this heading.

Our experiments do not allow any conclusions as to the driving force, for the displacement of dyestuff molecules from the surface of the enamel into its depth may be the result of the capacity for diffusion of these molecules and a flow of liquid. It may even be imagined that the rate of diffusion is greater than that of a flow of liquid, if there is one, directed to the surface of the enamel in consequence of which the molecules, in spite of the counter current, get lodged in the depth of the enamel. The short periods of coloring found by us will not provide a basis for any conclusions on this point until much further knowledge has been acquired, for example, about the relation between the sizes of the particles of dyestuff and the width of the so-called pathways. For a quantitative investigation we should know (a) the size of the particles, (b) the width of the pathways and (c) the nature of the molecules in the pathways. Besides, the problem is rendered more complicated by a contingent interaction between Trypaflavin and the contents or the lining of the permeable structures.

Therefore, one should not interpret a displacement of dyestuffs too rashly either as a flow of liquid or as a process of diffusion.

In the light of the above considerations it is evident that conclusions with regard to the physiology of the enamel based solely on the results of dyeing experiments must be considered with extreme caution. It is, of course, quite wrong to allow oneself to be led on the strength of such experiments to conclusions as to the vitality of enamel, even if enamel in vivo would behave in a different way from enamel in vitro (which in the present study has not been shown to be the case) as mere permeability does not prove the existence of protoplasm and it is only protoplasm that may be credited with vitality. Any justifiable conclusions as to the problem of enamel permeability as a whole cannot be reached until after many diverse experiments, the results of which are interpreted with due regard to the limitations of the experimental technic. The present study is intended as an attempt to this effect.

SUMMARY

Much of the confusion in literature concerning the permeability of enamel must be ascribed to the failure of many authors in observing the limitations of the methods they used.

First of all, therefore, we tried to divide the problem of permeability in several parts and we discussed the technical difficulties encountered if one tries to cover the problem in its entirety.

Next, the possibilities, and the limitations, of an investigation with dyes applied on unimpaired enamel were given.

The reason why Trypaflavin, a fluorescent dye, was chosen and the measures taken in order to prevent dislocation of the dye during the preparation of the specimens were discussed in detail.

The results were that Trypaflavin permeated unimpaired enamel of dogs' deciduous (and, to a somewhat lesser extent, permanent) teeth. It was found in the prism sheaths, the tufts, and the lamellae.

After an application of 2 minutes' duration of a ½ per cent Trypaflavin solution the dye was found to have permeated the whole depth of the enamel cap of a dog's deciduous canine.

It was stressed that, although this investigation did not yield new morphological data, it certainly proved for the first time that the above-mentioned structures of the enamel really can act as channels of permeability under normal circumstances.

Finally, some problems arising from but not solved by this investigation were discussed.

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THE GRINDING OF VERY THIN SECTIONS OF ENAMEL

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ALTHOUGH for many purposes the usual ground sections of enamel (0.150 to 0.050 mm. thick) are quite satisfactory, one is occasionally obliged to use very thin preparations (0.010 to 0.005 mm. or less) as in fluorescence or polarizing microscopic investigations.

The known technics (Bodecker,[1] Patten and Chase,[2] and Lefkowitz[3]) aimed at the preparation of large and complete rather than of extremely thin sections. The above-mentioned thinness of less than 0.01 mm. is not stated to have been attained. Lefkowitz[3] remarked that enamel ground to 0.02 mm. did not withstand handling.

Gustafson[4] prepared the very thin enamel sections he needed in his investigations by manually grinding off thick sections so as to render them wedge shaped. Apparently, only narrow strips of very thin enamel resulted from this technic.

The sections we prepared in the apparatus to be described below turned out to be wedge-shaped, but by refining the technic we succeeded in decreasing the slope of the wedge so as to produce relatively large areas of uniform thinness.

Fig. 1 depicts the principal parts of the apparatus used for this purpose. The rotating bronze lap (*a*) is charged with diamond powder on its upper surface. The object carrier (*b*) is moved slowly to and fro along the line *AB* exactly parallel to the surface of the lap. This movement ensures an even use of the lap. Both the rotating movement of the lap (1000 r.p.m.) and the reciprocating movement of the object carrier (5 strokes per minute) are brought about by one small electric motor.

Apart from the automatical reciprocating movement, the object carrier can be manually lowered and raised relative to the lap, the amount of displacement being readable in $\frac{1}{300}$ of a millimeter.

The bearings of the lap and the object carrier are attached to the knife holder and the object slide, respectively, of a slightly altered Minot microtome so as to make use of the sliding movement and the micrometer screw of the microtome for the purpose indicated above.

The part designated *c* is best described as an inverted hat of methyl methacrylate. It will be referred to as the "object," though the proper object (i.e., the section to be ground) is only part of it. The object is made from powder and liquid in a brass cuvette of the appropriate form, on the bottom of which the section is laid. After polymerization has taken place, the section will be found enclosed in the plastic material from all sides except the undersurface. The undersurface of the section will be parallel to the plane of the rim of the object if the corresponding parts of the cuvette have been accurately

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machined and if the section was really perfectly flat on the bottom of the cuvette. In that case the upper surface of the section will be parallel to the rim as well, provided the section is planoparallel.

The central part of the object passes through the hole in the object carrier. It projects for about 1 mm. beyond the lower surface of *b*, if the rim of the object rests on the three studs visible in Fig. 1. The object is gently pressed down onto these studs by a spring attached to *b*.

In Fig. 2 the parts *a*, *b*, and *c* may be seen in longitudinal section and the spindle of the lap and its bearings are shown diagrammatically.

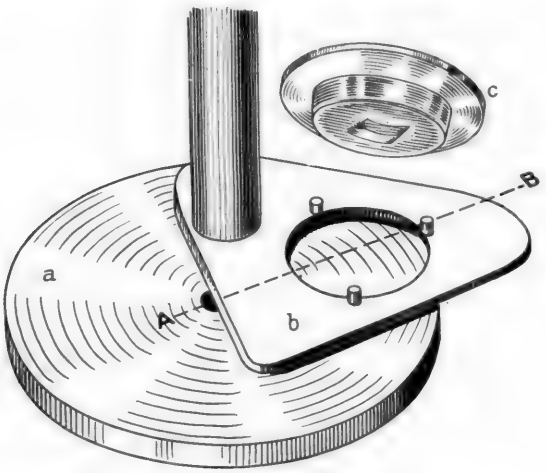

Fig. 1.

The grinding process proceeds as follows: The section to be ground is enclosed in an object as described above. In this step the section is provided with a hard supporting layer in order to keep intact even the thinnest parts of the brittle material during the grinding process. Considerable difficulty was encountered in securing a material which, besides being hard enough, could be made to adhere to the surface of the section. A formaldehyde resin was advised but proved to be too brittle, though adhesion to the section was good. Another type, being very hard and adhesive, did yield good results, but the high processing temperature (from 130° C. upwards) is a serious drawback. Moreover, difficulties are encountered in eliminating this plastic from the finished section, the former being almost insoluble.

Eventually we found that methyl methacrylate did fairly well at low grinding speeds under very gentle pressure, although no actual adhesion of this type of plastic to the surface of sections can be expected. Probably the adherence of the sections to objects made from methyl methacrylate can be accounted for by the grip of the latter to minute crevices and ridges in the section. We found that enamel is held somewhat more firmly than dentin.

After the section has been embedded the object is placed, the section facing downward, on the studs and the apparatus is started.

By means of the micrometer screw the object carrier is now lowered onto the lap. As the lowering of the object carrier is continued over a known distance after the object touches the lap, the rim of the object will be lifted from the studs to the same amount. Grinding now proceeds under the pressure of the spring over the above-mentioned distance until the rim again rests on the studs.

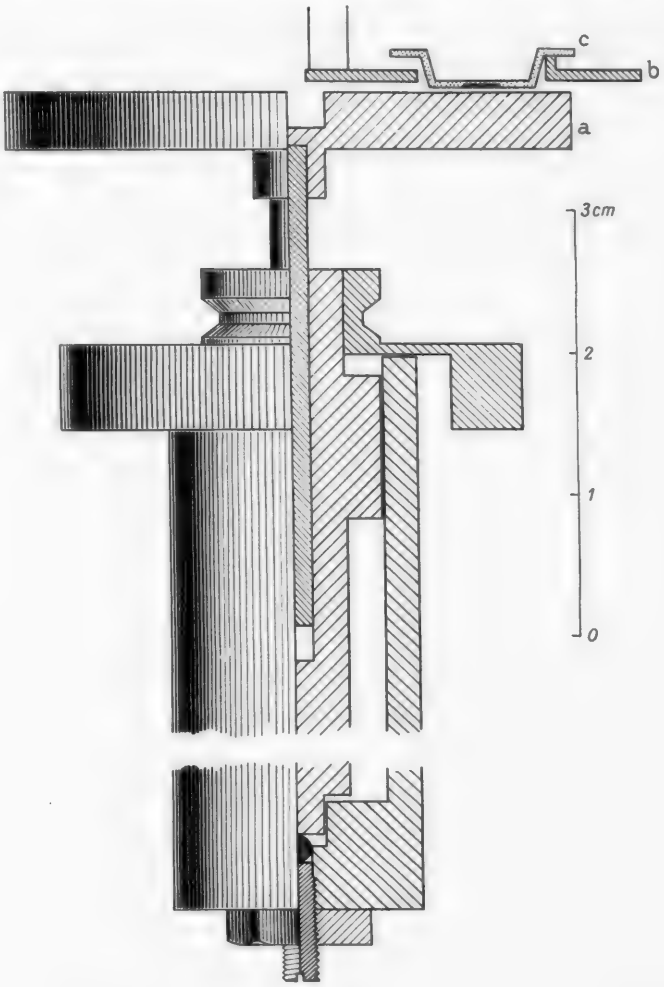

Fig. 2.

If microscopic inspection reveals that the section should be ground off further, the process is repeated, the amount of grinding being gauged according to the microscopic findings. Microscopic control of the grinding process is essential and it is rendered possible by the special construction of the object and object carrier, which makes for an easy removal and an accurate replacement of the

object. If even thinness is required over the entire surface of the sections, the following points should be observed: (1) the studs should be in a plane accurately parallel to the surface of the lap, and (2) the upper surface of the sections should be perfectly flat and parallel to the rim of the object.

The latter condition can only be partially fulfilled in sections sawed with a circular saw, as these sections cannot be considered planoparallel. Consequently, these sections will show thin and thick parts when ground as described. Of course, one may try to render the sections exactly planoparallel prior to grinding, but it is believed that for practical purposes the disadvantage of extra manipulation of the sections is not compensated for by the gain in the quality of the resulting thin section.

In one particular investigation of permanent and deciduous dogs' teeth, we applied this method and obtained enamel sections in which the thickness ranged from 0.005 to 0.030 mm., the thinnest parts having an area of approximately 0.1 by 0.1 mm. Attempts at grinding dogs' enamel thinner than 0.005 mm. mostly resulted in cracked areas from which the enamel was partly lost. In some instances, however, small areas of intact enamel of 0.002 or 0.003 mm. thickness could be observed. Dentin can be ground even thinner; the thickness of the thinnest parts could not be measured to any degree of accuracy by means of the micrometer screw of the microscope.

As in this particular investigation we could allow only the most essential manipulations of the sections, these data apply to sections ground after being sawed without any attempt at polishing or planing.

It will often be found desirable to remove from the ground sections the methyl methacrylate in which they are embedded. However, as the very thin parts of the section are easily damaged, a special technic was adopted: the object is glued, with the section downward, to a slide by means of a cellulose glue. Afterwards the methyl methacrylate is dissolved away in chloroform, which does not dissolve the cellulose. The result is a very thin section, freed from methacrylate, adherent to a slide.

SUMMARY

A technic is described enabling the preparation of ground enamel sections of which, in areas of approximately 0.1 by 0.1 mm., the thickness is 0.005 mm. and sometimes even less.

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THE PREVALENCE, DISTRIBUTION, AND MORPHOLOGIC CHANGES OF THE EPITHELIAL REMNANTS IN THE MOLAR REGION OF THE RAT

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INTRODUCTION

THE PURPOSE of this study is to classify the different forms of epithelial remnants and to investigate the prevalence and incidence of these structures in the periodontal membrane of the molars of the rat. This study is an attempt to provide a base line for later investigations on the fate of the epithelial remnants of the rat molars under different systemic conditions and to serve as a part of a broader study of the biology of epithelium.

REVIEW OF THE LITERATURE

Malassez (1885, *a*) was the first to describe remnants in the periodontal tissues. He seems to have recognized the origin of epithelial remnants from Hertwig's epithelial root sheath. Since he also described, under the term "debris epitheliaux," groups of epithelial cells located between the unerupted crown and oral mucosa derived from the dental lamina, it appears not quite justified to connect his name with the epithelial remnants of the periodontal membrane alone. Malassez (1885, *b*), however, stressed the significance of the epithelial remnants in the etiology of epithelial cysts.

The studies of von Brunn (1887) on the development of the roots of rat molars clarified the function of the epithelial sheath and the development of the epithelial remnants. von Brunn showed that the epithelial root sheath consists of the inner and outer enamel epithelium only, that it is the determining factor in the shaping of the root and in the differentiation of the connective tissue cells of the pulp into odontoblasts, and that after it is perforated by the connective tissue of the primitive periodontal membrane, it gives rise to the periodontal epithelial remnants.

Black (1887) considered the epithelial remnants first as lymph canals which are lined by epithelium and filled with lymph. Later (1899), he interpreted these structures as ductless glands which show a netlike arrangement around the tooth.

Lartschneider (1929) claimed that the epithelial remnants originating from dental lamina give rise to multiloculated cysts and enamel-forming tumors, whereas the epithelial remnants originating from the epithelial root sheath might develop into simple cysts.

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Part of a dissertation submitted by F. M. Wentz in partial fulfillment of the requirements for the degree of Master of Science in Histology at the University of Illinois.

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Orban (1929) found epithelial remnants in the bony canals containing the dental arteries, veins, and nerves, in the pulp, and in the interdental alveolar septum.

Epithelial remnants are of regular occurrence in the periodontal membrane (Orban, 1929, Fischer, 1932, Meyer, 1932). Meyer found that epithelial remnants show in younger individuals a netlike arrangement. Fischer observed this arrangement rarely in human teeth but regularly in the dentition of herbivora. He stressed the frequency of epithelial remnants at the apex, the bifurcation, and the cervical region of the tooth. The cells, which he considers without function, have a more or less pyknotic nucleus and a small amount of cytoplasm. Sometimes one is able to distinguish the two layers of the epithelial sheath. Meyer found that the epithelial remnants are located close to the tooth surface within the bundles of the principal periodontal fibers and not in the spaces between the bundles.

Robinsohn (1925) suggested that the epithelial remnants have an endocrine function.

Noyes, Schour, and Noyes (1938) in their textbook state that epithelial remnants may degenerate, become calcified, and give rise to cementicles or in inflammatory processes may lead to root cyst formation. The epithelial remnants are more numerous in the young than the old, but may persist throughout life. In one instance they were demonstrated in the periodontal membrane of a man 70 years of age. The authors do not ascribe any particular function to the epithelial remnants but regard it as unlikely that embryonal debris would persist throughout life if it served no function.

MATERIALS AND METHODS

The material for this study consisted of histologic sections of the molars of 105 rats. The animals constituted part of the wistar age study. Different organs and tissues were sent to various investigators throughout the country for study. The jaws and teeth were sent to the Department of Histology of the University of Illinois College of Dentistry for histologic preparation and analysis.

The animals comprised a series between the ages of 75 and 1,088 days. The group between 75 and 300 days of age consisted of 38 albino rats; the group between 300 and 1,088 days consisted of 35 albino and 32 gray Norway rats. The rats were fed on Purina Dog Chow ad libitum.

The jaws were fixed in 5 per cent formalin. They were decalcified in 5 per cent nitric acid and embedded in celloidin. Molars and incisors were sectioned in one block in an anteroposterior direction. The sections were stained with hematoxylin and eosin.

FINDINGS

A. Morphology of the Epithelial Remnants

Three different morphologic types of epithelial remnants could be distinguished: (1) small resting type, (2) proliferating type, and (3) differentiating type.

Fig. 1.

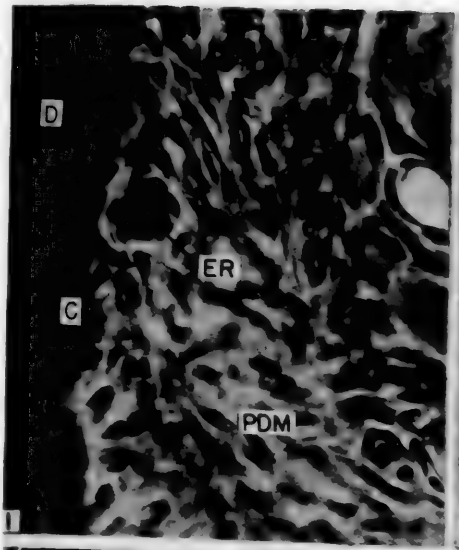

Fig. 2.

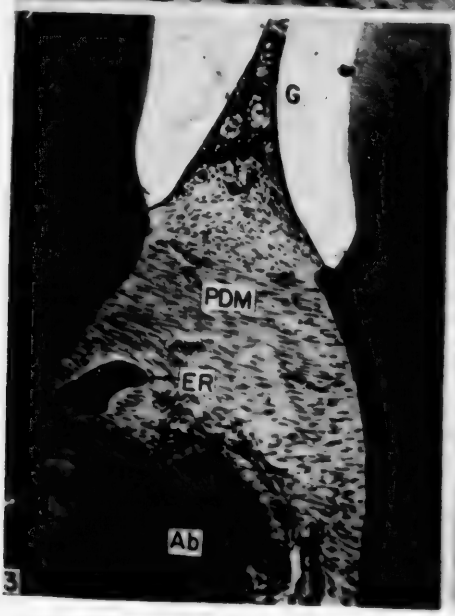

Fig. 3.

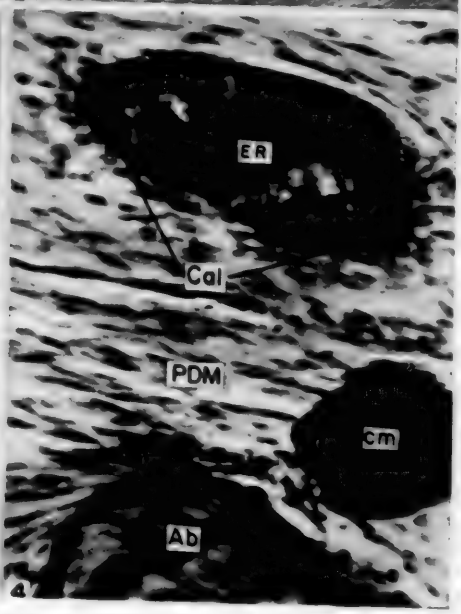

Fig. 4.

Figs. 1 to 12.—Key: *Ab*, alveolar bone; *B*, bifurcation; *C*, cementum; *Cal*, calcified area; *Cm*, cementicle; *D*, dentin; *EAB*, budding proliferation of epithelial attachment; *EB*, budding proliferation of gingival epithelium; *ER*, epithelial remnant; *F*, fracture of root; *G*, gingiva; *P*, epithelial pearl; *PDM*, periodontal membrane; *Rab*, resorption alveolar bone.

Fig. 1.—Photomicrograph showing small resting type of the epithelial remnants. (WA295) Age, 75 days. Magnification ×650.

Fig. 2.—Photomicrograph showing a small resting type epithelial remnant arranged in a strand. Tangential section of cementum crypt. (WA232.) Age, 100 days. Magnification ×300.

Fig. 3.—Photomicrograph showing proliferating type of the epithelial remnants, larger form. (WA232.) Age, 100 days. Magnification ×120.

Fig. 4.—Photomicrograph showing the proliferating type of the epithelial remnants, larger form. Partial calcification has taken place. (WA737.) Age, 700 days. Magnification ×440.

1. *Small Resting Type* (Fig. 1).—The small resting type of epithelial remnants had an average diameter of 30μ and were found close to the cementum. They consisted of an average of 7 to 10 cuboidal cells which were arranged in spherical groups.

Some variations in the location of the nuclei of the cells could be observed. In some instances the nuclei of the peripheral cells were lying at the basal end of the cells. In other instances they were located at the distal end of the cells. In the latter case the periphery of the epithelial remnant was formed by the cytoplasm of the cells, and the nuclei appeared crowded toward the center of the epithelial remnants. In some instances the nuclei appeared pyknotic.

The connective tissue surrounding the epithelial remnants also showed variations. In most instances the connective tissue adjacent to the epithelial rests showed hyalinization. In other instances the fibroblasts were arranged in an onion-leaf manner.

On tangential section, the cells of the epithelial remnants appeared in a strand arrangement (Fig. 2). A study of successive sections revealed that the structures were tortuous and communicated with other epithelial remnants in the vicinity. The strands were of varying diameter and averaged 15μ. The cuboidal cells contained ovoid nuclei. Two to five cells could be counted in a diameter of the strand.

In the vicinity of some of the strands an irregularity in the contour of the cementum was noted.

2. *Proliferating Type.*—The majority of this type of epithelial remnant was observed close to the cementum and averaged twice the size of the small resting form. In some instances the cementum tended to include the remnants.

Two variations of this form were observed:

(a) *Smaller form.*—This form consisted of an average of 35 to 40 cells with small ovoid nuclei. Each structure varied between 60 to 75 days μ in diameter. The capsule was thick and stained intensively with eosin. Closely packed hyalinized fibers were distinguished in the capsule.

An incidental finding in a 300-day-old animal showed this type of epithelial remnant in the pulp of a molar. It was situated close to the dentinal wall. The odontoblasts had completely disappeared in this area and the capsule consisted of a structureless, uncalcified matrix. A study of successive sections demonstrated a near-by accessory root canal which might be considered as the probable mode of entry of these epithelial cells into the pulp.

(b) *Larger form* (Fig. 3).—This form was composed of 50 to 100 cells with large, well-formed nuclei. The average diameter varied between 75 and 150 μ and was usually observed in the interdental space and in the cervical third of the molars.

One animal, 700 days of age, showed partial calcification of this form of epithelial remnant (Fig. 4).

3. *Differentiating Type.*—The differentiating type of the epithelial remnants consisted of ovoid islands of cells and averaged 390 μ at the broadest diameter (Fig. 5).

A distinct difference in cellular morphology and staining reaction was noted between the peripheral cells and the cells of the central area. The peripheral cells were cuboidal and arranged regularly on a basement membrane. They resembled a stratum germinativum of stratified squamous epithelium. The scanty cytoplasm stained more intensively than that of the central cells. Because of the scarcity of the cytoplasm of the peripheral cells the nuclei appeared closer together than in the central area of the structure. Peculiar, characteristic, deeply staining striations were seen at varying intervals but were limited to the peripheral area. Every example of the differentiated type of epithelial remnant presented this feature. The cells of the central portion were more loosely arranged and generally had smaller nuclei with oval to spherical shape.

The differentiated type was found singly or in groups of two to four. However, in one instance an animal of 300 days showed a group of 10 islands close together (Fig. 6). The resemblance to glandular acini was marked in some instances. Clefts are found in some islands.

In 6 cases the differentiated type occurred deep in a cementum crypt in the bifurcation of the first molar. In these cases the capsule appeared thicker than in those which were found free in the periodontal membrane. The adjacent acellular cementum may encapsulate the remnant almost completely. Study of successive sections indicated the probability that in some instances the remnants in the bifurcation were at the opening of root canals.

B. The Prevalence of Epithelial Remnants

The periodontal membrane of the molars of 53 (approximately 50 per cent) of 105 specimens showed the presence of epithelial remnants.

Sex was not a factor in the prevalence of the epithelial remnants. Thirty-one of 60 females and 22 of 45 males demonstrated the epithelial structures. The gray Norway rats showed a greater prevalence of specimens with high remnant counts than did the albino rats. A comparison of animals between 700 and 1,088 days showed 7 of 30 Norways and 2 of 34 albinos with high counts.

The small resting type and the proliferating type occurred with the same frequency in the albino and in the gray Norway rats. The differentiating form, however, showed greater prevalence in the gray Norway rat. Twelve of 105 animals demonstrated this form; 7 of 30 gray Norway rats, and 5 of 75 albino rats.

C. The Incidence of Epithelial Remnants

The incidence of epithelial remnants decreased with increasing age. This finding was noted after the specimens had been divided into three age groups (each group consisting of approximately the same number of animals):

Group 1.—(75 to 300 days of age.) Twenty-seven of the 38 specimens showed epithelial remnants (approximately 71 per cent).

Group 2.—(301 to 700 days of age.) Fourteen of the 31 animals demonstrated remnants (approximately 45 per cent).

Group 3.—(701 to 1,088 days of age.) Twelve of the 36 animals had epithelial remnants (approximately 33 per cent).

A study of the incidences of the different types of the epithelial remnants showed that the proliferating type constituted the majority of the epithelial remnants and was fairly constant from 75 to 1,088 days. The small resting type decreased and the differentiating type increased with increasing age.

Fig. 5. Fig. 6.

Fig. 7. Fig. 8.

Fig. 5.—Photomicrograph showing differentiating type of the epithelial remnants. (WA74.) Age, 150 days. Magnification ×88.

Fig. 6.—Photomicrograph showing differentiating type of the epithelial remnants in group formation. (WA24.) Age, 300 days. Magnification ×88.

Fig. 7.—Photomicrograph showing extreme septal atrophy (*Rab*) associated with root fracture (*F*). The differentiating type of the epithelial remnant may be seen in the bifurcation of the first molar (*B*). (WA22.) Age, 878 days. Magnification ×13.

Fig. 8.—Photomicrograph showing enlargement of (*B*) bifurcation of first molar of Fig. 7. Differentiating type of remnants may be seen in bifurcation. (WA22.) Age, 878 days. Magnification ×105.

Fig. 9.

Fig. 10.

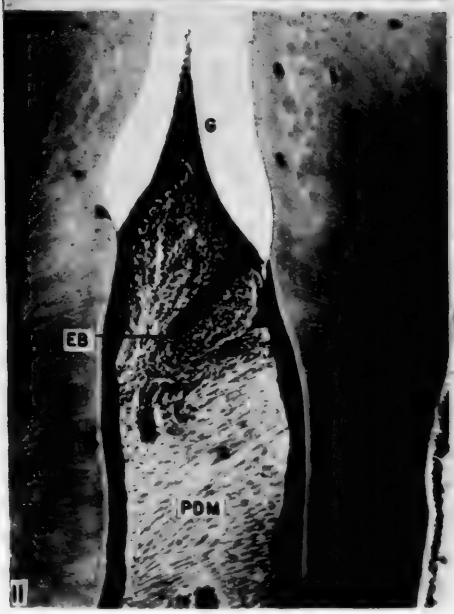

Fig. 11.

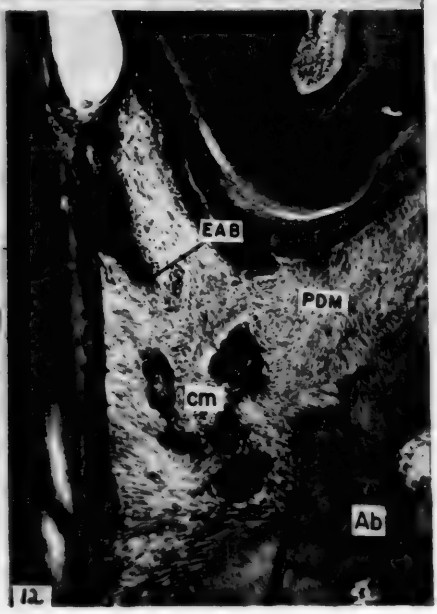

Fig. 12.

Fig. 9.—Photomicrograph showing differentiating type of epithelial remnants with epithelial pearl-like formation. (WW168.) Age, 333 days. Magnification ×105.

Fig. 10.—Photomicrograph showing enlargement of (*P*) epithelial pearl-like formation in Fig. 9. (WW168.) Age, 333 days. Magnification ×650.

Fig. 11.—Photomicrograph showing budding proliferation of gingival epithelium (*EB*). (WA8A.) Age, 700 days. Magnification ×105.

Fig. 12.—Photomicrograph showing budding proliferation of epithelial attachment (*EAB*). Cementicles are found in periodontal area below epithelial attachment. (WA1002.) Age, 1004 days. Magnification ×105.

E. Location of Epithelial Remnants

The majority of the epithelial remnants were found in the supra-alveolar areas of the molars of the albino as well as of the gray Norway rats (Fig. 13).

1. *Supra-alveolar Area.*—This was the area above the normal alveolar crest of the molars and just below the cemento-enamel junction. It contained nearly one-half of all the epithelial remnants, 47 per cent of the total in the albino rats and 44 per cent of the total in the gray Norway rats.

2. *Midroot Area.*—This area was defined as the region of the middle third of the root. It contained 30 per cent of all the remnants in the albino series and 22 per cent of these structures in the grey Norway series.

3. *Bifurcation Area.*—This area was the region between the bifurcation of the roots and above the normal alveolar crest. It contained 15 per cent of the remnants in the albino series and 26 per cent of the remnants in the grey Norway series.

4. *Apical Area.*—This area was in the apical third of the root and demonstrated 8 per cent of the total in the albino rats and 5 per cent of the total in the gray Norway rats.

DISCUSSION

Resting Type.—This form was found arranged either in strands or in small islands. The strands were found in tangential section in animals varying in age up to 300 days. We may assume that the small islands were cross sections of the strands.

This type decreased with increasing age. This might have occurred by (a) disintegration and resorption, (b) degeneration and consequent calcification into cementicles, or (c) change into the proliferating type.

Proliferating Type.—This was the most frequent type, and it probably represents the stage in which the epithelial remnants survived longest. It may degenerate and disappear in a manner similar to the small resting type, or, under the influence of some factor, may start to proliferate and transform into the differentiating type. The influencing factor was probably irritation. This could be borne out by the fact that all the larger forms of this type were in the supra-alveolar area, a region where the degree of irritation was high.

Differentiating Type.—The differentiating type of epithelial remnants increased with age and in all instances occurred in either the bifurcation area or the supra-alveolar area. In every circumstance where this type was found, the interdental septum was resorbed to a varying degree. Shepro (1947) noted that the septal atrophy in the rat is a physiologic process associated with age.

It may be assumed that the physiologic loss of suspensory apparatus leads to increased mobility. Figs. 7 and 8 show an example associated with the loss of the alveolar septum where mobility had been so extreme that the distal root of the third molar had fractured. The epithelial remnants may be distinguished in the bifurcation of the first molar.

The increased mobility might furnish a stimulating factor for the change of the proliferating type into the differentiating type. This type in the greater majority showed two differentiated forms of cells. In one instance (Figs. 9 and 10), a third cell form was demonstrated in an epithelial pearl-like formation. Numerous mitotic figures and a wide capsule surrounding the structure were also noted.

Some observations were in disagreement with the preceding interpretation. In a number of animals 700 days and older, portions of the gingiva showed areas of budding proliferation extending into the surrounding connective tissue (Fig. 11). These buds may become isolated into islands and simulate the epithelial remnants.

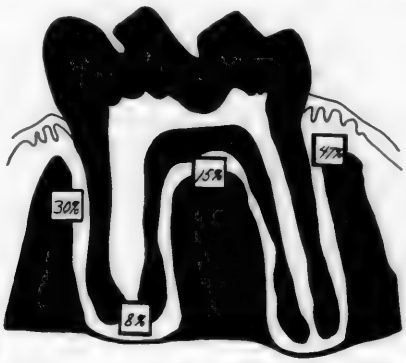

Fig. 13.—Location and percentage distribution of epithelial remnants in albino rats 75 to 1008 days of age.

The Epithelial Attachment as Possible Source of Epithelial Proliferation.—It is sometimes difficult to decide whether an island of epithelial cells located in the cervical portion of the periodontal membrane originated from Hertwig's epithelial root sheath or from the epithelial attachment. The cementicles seen in Fig. 12 might be calcified epithelial remnants or a calcified isolated portion of the proliferated epthelial attachment. The assumption that some of the epithelial remnants have developed from the epithelial attachment would explain the strange fact that the number of epithelial remnants is greatest in the cervical area of the root in spite of the fact that it is the oldest portion of the root.

SUMMARY AND CONCLUSIONS

This investigation is based on the histologic study of the supporting tissue of the molars of 105 normal male and female albino and gray Norway rats ranging in age from 75 to 1,088 days.

1. Epithelial remnants were found in approximately 50 per cent of the animals.

2. The incidence of the remnants decreased with increasing age.

3. Three morphologic types were noted: (a) a small resting type, which decreased in number with increasing age; (b) a proliferating type, which comprised the majority of the epithelial remnants, and (c) a differentiating

type which increased in number with increasing age and was correlated to resorption of the alveolar septum.

4. The majority of the epithelial remnants were found in the supra-alveolar area above the normal alveolar bone (Fig. 13).

5. It may be assumed that the three morphologic types represented successive stages in the life history of the epithelial remnants. The small resting type upon proliferation became transformed into the proliferating type. The latter, particularly when stimulated by traumatic factors, may differentiate into the third type.

6. While the great majority of epithelial remnants probably are derived from Hertwig's epithelial root sheath, some might originate from the epithelium of the gingiva or from the epithelial attachment.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the Philadelphia Section

University of Pennsylvania School of Dentistry, May 15, 1950*

Compiled by S. Leonard Rosenthal, *Editor, Philadelphia Section*

1. Herpetic Stomatitis. *T. McNair Scott, Children's Hospital, Philadelphia, Pa.* Most frequently found in children from the sixth month to the sixth year, it is not uncommon in adult life. Laboratory diagnosis may be made by transfer of the contents of a vesicle to the cornea of a rabbit's eye or by microscopic examination of scrapings prepared with Giemsa stain. The presence of "inclusion bodies" in the cells differentiates herpetic from recurrent aphthous stomatitis. Infection by the herpes virus induces rapid antibody formation. No antibodies were found in the blood following attacks of recurrent aphthous stomatitis.

2. Oral Manifestations of Disorders of the Blood. *Leandro Tocatino, Jefferson Medical College, Philadelphia, Pa.* The mouth plays an important role in diagnosis since the first manifestations of blood dyscrasias may be found there. The simplest manipulations of the oral mucosa may be dangerous, and even the toothbrush may induce hemorrhage.

3. Organogenesis in Tissue Culture. *C. E. Wilder, Jr., University of Pennsylvania Dental School, Philadelphia, Pa.* Portions of the salamander embryo were used to determine whether organs can form themselves without influence of the entire embryonic mechanism. To obtain organotypic growth requires an epithelial sheath, otherwise histotypic growth will result. The gill and the leg developed in tissue culture. The extent of development was dependent upon the stage of the embryo when the part was removed, but no complete development was observed. There seems to be an integrating mechanism in the embryo which is essential for complete and proper development of its parts.

*Ernest Ritsert, presiding.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section*

The Guggenheim Dental Clinic—May 4, 1950†

Compiled by Frances Krasnow, *Editor, New York Section*

1. The Range of Histological Variation of Clinically Normal Gingivae. *Leo Zack, Dental Department of Montefiore Hospital. New York, N. Y.* (Abstract published in *J. D. Res.* **29**: 699, 1950.)

2. A Study of the Cornification of the Oral Epithelium by the Use of Oral Scrapings: A Preliminary Report. *S. C. Miller, A. Soberman, and S. S. Stahl, College of Dentistry, New York University, New York, N. Y.* (Abstract published in *J. D. Res.* **29**: 678, 1950.)

3. In Vivo Study of the Uptake of Radioactive Iodine by Human Teeth. III. *Herbert J. Bartelstone, Dental Department of Montefiore Hospital, New York, N. Y.* (Abstract published in *J. D. Res.* **29**: 684, 1950.)

4. Modifications of Mandibular Architecture Following Removal of the Temporalis Muscle in the Rat. *Sidney L. Horowitz, Research Assistant in Dentistry, School of Dental and Oral Surgery, Columbia University, and Harry H. Shapiro, Department of Anatomy, College of Physicians and Surgeons, New York, N. Y.* Gross findings and grenz ray studies following the unilateral removal of the temporalis muscle in the young rat show changes in the form and architecture of the mandible. The coronoid process fails to develop on the operated side. Examination by grenz ray of both the operated and control sides of the mandible indicate changes in the direction and formation of the bony trabeculae.

5. Effects of Temperature on Salivary Amylase Activity. *Leon H. Schneyer, Dental Department of Montefiore Hospital, New York, N. Y.* (Abstract published in *J. D. Res.* **29**: 696, 1950.)

6. Group Variation in the Prevalence of Unerupted Permanent Maxillary Canines. *Eli H. Siegel and Irwin Ferber, North-Eastern Dispensary, New York, N. Y.* Complete radiographic and clinical examination of 990 recently emigrated Jewish displaced persons showed a prevalence of 4.6 per cent of either 1 or 2 unerupted maxillary canines. Fifteen of 498 males and 31 of 492 females were affected with a prevalence, respectively, of 3.0 per cent and 6.3 per cent. These persons originated almost entirely from the Balkans, Poland, Germany, Austria, Czechoslovakia, the Ukraine, and the Baltic countries, and were all of posteruptive age for the canines. A control series of 464 non-Jewish American and European whites of wide geographical origin showed a frequency of 1.5 per cent; males, 1.2 per cent and females 2.2 per cent. Data in the literature show frequencies similar to those reported for this group. This condition thus appears twice as often among females than males in most groups. The prevalence of unerupted maxillary canines among European Jews is greater than reported for non-Jewish European whites, American non-Jewish whites, American Indians, African Negroes, Chinese and Alaskan and Greenland Eskimos. Preliminary data indicate a very low frequency among American Negroes. These findings are ethnic variations of possible hereditary origin and, as such, are of anthropological significance.

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*The cost of publication of these proceedings is paid by the New York Section.
†Gerrit Bevelander, presiding.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Proceedings of the Twenty-Eighth General Meeting

French Lick Hotel, French Lick, Ind.

March 24, 25, and 26, 1950

Compiled by Hamilton B. G. Robinson, Editor, *College of Dentistry Ohio State University, Columbus, Ohio*

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I. Inaugural Address: The Scientific Emphasis in Dentistry

B. G. Bibby, *Rochester, N. Y.*

To be elected to the presidency of the International Association for Dental Research is an honor for any man. I am deeply aware of that honor and most grateful for it. In accepting this office, my feelings of gratitude are enriched by sentimental attachments which I have felt for this organization since I first came to be associated with it. As a dental stranger in a strange land with no long-standing friendships and without any support or encouragement from any dental organization, it was an important occasion when, on attending my first meeting of this Association some eighteen years ago, I found amongst its members a body of men who understood and appreciated the scientific and educational objectives which had led me into the then small and almost suspect field of dental research. To one who was born and educated in a foreign land, the word "international" in our title was particularly significant and it was honorably exemplified by the membership of those days. Probably largely as a result of my own fortunate experiences, I have always felt that central purposes of this organization should continue to be the encouraging of young men who are trying to make a career in research and the building of scientific and personal bridges across the abysses of international misunderstandings. Since my conviction of the worth of these purposes is founded on an interaction of reason and sentiment, it is easy for me to pledge my services to the furtherance of the objectives of our organization.

In this Association there seems to be no binding precedent as to subject or nature of the remarks which your new president has to make. It may be as-

The meetings of the International Association for Dental Research preceded the meetings of the American Association of Dental Schools at the same hotel on March 27, 28, and 29.

The Editor was given valuable assistance in the preparation of these Proceedings by the General Secretary, Edward H. Hatton.

sumed, therefore, that he is entitled to the privilege of abandoning the rigid rules of scientific caution and talking on the subject of his choice. I plan to exercise this prerogative to discuss what seems to me to be a changing attitude of mind concerning the place of science in the life of man.

* * * * *

Although dental circles have so far been little concerned about it, other scientific organizations have been devoting an increasing number of scholarly discussions to considerations of the social implications of the present day emphasis on science and research. Under the title "Science Can Be Silly" the subject has recently been brought to public attention in the news magazine, *Time*. In spite of the questionings of a few scientists as to whether scientific progress had a purposeful or desirable direction, it is only recently, and then largely as a result of the explosion of the atomic bomb, that it has become quite respectable to raise such doubts. The leer of atomic death has given an air of desperate urgency to the old problem of the place which science should occupy in the affairs of mankind.

The conflict of ideas between Science, has represented first by agriculture and astronomy and later by physics and biology, and the Humanities as represented by social custom, religion, and the arts, is not new. There was never a period in the history of mankind in which one set of ideas was not in conflict with another. It is improbable that Nomadism was supplanted by Agriculture, the most primitive of scientific pursuits, without acrimony and bloodshed. The customs and belief which discouraged Galen from human dissection or which buttressed belief in a geocentric universe are but further evidence of man's basic biological disinclination to abandon a relatively satisfactory present for an uncertain future.

While scientific advance was slow, modest adjustments in the cultural and mental attitudes of men made it possible for them to preserve for themselves a satisfying fabric of life. Those who wished could follow scientists into new lands, new habits of life, or new worlds of thought, and the others, always the bulk of mankind, could continue almost undisturbed in the ways of their fathers. But even then there were those who doubted whether science's influence was for the best, and in 1815 we find Thomas Jefferson lamenting that "morals do not of necessity advance hand in hand with the sciences." When, as in recent times, the rate of scientific discovery accelerated so rapidly, most of the reservations regarding its benefits were blotted out. Professional and amateur scientists alike saw visions of brave new worlds into which the scientists would lead mankind if mankind would but have the wisdom to follow. If further persuasion was needed, it was supplied by the dramatic results of industrial applications of scientific discovery and the glamorous promises of imaginative newswriters. Had not science shown that disease was bacterial in nature and not of spiritual origin? Had it not revolutionized the physical aspects of daily life and discredited traditional theology? It is little wonder that almost everybody came to believe that scientists would soon produce a push button paradise replete with panaceas for poverty and pills for psychoses. Science could discover all and order all, and Hippocrates' idea that man was the measure of all things was obviously out of date.

As propagandists are often victims of their own propaganda, many scientists were deceived by the mirages they had helped to create. While a minority of scientists worried about the social impact of their discoveries, the majority felt that their only responsibility was to add more speed to the pace of scientific discovery. Encouragement and respect from those who were mystified, liberal treatment by those who held the purse strings, an enshrouding sense

of purpose in his work, and gratification arising from pursuit of his own interests made the scientist something of a modern aristocrat. There began to appear an aristocracy of science almost as irresponsible in regard to the spiritual or emotional needs of the people as were the propertied aristocrats of earlier times of the bodily needs of their inferiors. Neither was there the sense of responsibility nor the noblesse oblige of the older order to take the curse off the situation. Because the emotional reactions of man could not be measured or controlled, they were, therefore, unworthy of the scientists' attention. Was it not his responsibility to leave alone those things which he did not undertstand and in which he had received no training and to which, he, therefore, could not apply quantitative scientific techniques? That stuff was for lesser breeds: novelists, practical psychologists, parsons, and other unworthy dabblers with the emotions—such as Adolph Hitler!

To douse the resultant emotional orgy—and that is what it was—science exploded the atomic bomb. In the quiet that followed that blast, scientists have everywhere paused to think and to question. Does science and scientific discovery have the power to save the world? Is it not as much the scientists' duty to recognize the limitations of science as a whole as it is to recognize the limitations of an individual experiment? Is not the proper utilization of discovery as well as the joy of making it a reasonable responsibility of science? Has not science thus far been destroying the beliefs, the idols, and the gods which had comforted the mass of the people, and in their place offered nothing which they could follow? Since, to paraphrase the Scriptures, it was as the result of science that many had stumbled and fallen, were it not better that a millstone was hanged about its neck and it were cast into the depths of the sea? Most thoughtful scientists admit at least partial guilt and scientific leaders are making sure that in the future science will avoid past errors and will serve a broader and even more useful part in the progress of mankind. Science, in short, has become maturely modest.

This, I believe, marks an important stage in the evolution of scientific thought, one which will mark the beginning of the unification of the forces of Science and the Humanities: those forces which are helping man to live longer and those which are helping him to live more happily. We are at the dawn of an important era.

* * * * *

What bearing does the changing emphasis in scientific thought have on dental research? Is there any reason for thinking that dental science will not be affected? Because it is small and young, because it never swayed man's imagination and because, until recently, it was almost entirely integrated with patient service, it never lost contact with the affairs of man and certainly it did little to disturb his pattern of life. Any effects which dental science can have had on mankind in general must have been of a very minor and indirect sort. It is only in the dental profession itself that one could expect dental science to exercise any real influence on individual or collective thought, and it is there we must look to see how well it has done in the past or how well it is likely to measure up to the future.

Dental science has been growing rapidly. At this, its adolescent stage of development, it has no very definite personality. It is not too soon, however, to look for signs of the character traits of its elder scientific brothers and, in view of the critical times in which it is growing up, it is not too soon to set about correcting any discernable deficiencies. If we are going to build the best future for scientific dental research, research workers cannot do better than to examine critically their motivations and attitudes. Let us do some soul searching.

As a beginning it might be well to ask why it is that we do scientific research. There is, of course, no single reason. Some do research because they like institutional life, some to earn graduate degrees, specialty qualifications, or professional recognition. Others offer the classic reason of the necessity for discovering new facts, which facts will presumably contribute to the advancement of dentistry and the world in general. All these purposes are laudable, but when we realize that research institutes will always be more efficient in the discovery of new information, they only partly justify rsearch activities in teaching institutions. Research in teaching institutions is beyond question only if it is making an educational contribution to either the staff or the student. It should convey to everybody who comes in contact with it an understanding of the limitations of existing knowledge, a comprehension of the advances that are taking place, and a realization of the interrelation which exists between apparently diverse fields of learning. Moreover, it should be borne in mind that this contribution can well be made completely independently of the discovery of any new facts.

Because most of the research activities in our profession are carried out by workers who have already received graduate degrees or gained professional recognition, a better explanation than that given above is needed to account for the continued research activity of so many persons. The reason that so many of us continue to devote time to research is very simple and basic. We do research because we want to do research. While we might like to hide this fact from ourselves with protestations of service to humanity or to our profession, I am reasonably sure that most of us continue to do research principally because of the emotional satisfaction we get out of it.

In considering whether dental scientists have an inclination toward development of an expanded ego, or toward expecting too much in the line of recognition and rewards for their research activity, the fact that most of us do research because of the satisfaction we get out of it is a basic consideration. If research and its related activities is our principal hobby, we deserve no special credit for additional years of study or long hours of work on it. We are simply lucky, or perhaps wise, because our hobby and our work is almost identical. We should expect to be entitled to no automatic distinction or given any privilege of authority because we have been doing research. The right to leadership is generally earned by distinguished achievement and sacrifice of immediate personal interests to group needs. Only when research workers have met these criteria should they expect the rights of leadership in their profession. Fortunately for dental research, few dental scientists have sought omnipotence and the ambitions of those few have not been aided by a dramatic background of scientific discovery in dentistry. The dental profession has not, therefore, been much influenced by the doctrine of self-appointed scientific Messiahs.

The sense of professional or social responsibility of dental scientists is most likely to be revealed in their institutional relationships. When research workers are provided with the opportunity to pursue their interests and to advance themselves by a teaching institution, they should recognize their responsibility for furthering its basic purposes. This can be done by contributing to its breadth of educational understanding and by placing emphasis on the many educational by-products of research. If dental research workers fail to do this and become so intent in pursuing their own ideas that they cease to try to make these educational contributions, then they are guilty of irresponsibility to their institution and their profession. Then they would seem to be beginning to travel the path of those scientists in other fields who by pursuing their own interests to the exclusion of all else have contributed towards the present critical attitude towards scientific discovery.

The same overemphasis on scientific discovery may have started to have a negative effect in other areas of dentistry. It has probably contributed to the concept that scientific discovery could solve all the problems of the profession, if not of the world. By preaching change and having too much confidence in what was "scientifically" new, we may have encouraged the idea that the past has no lessons for today. By acting as if it was the speed of change, rather than its direction which was important, we may have contributed to professional and social instability. By allowing our scientific activities to be overpublicized we may have helped to overshadow technical and intellectual achievement in other fields and helped to force into the background nonscientists whose scholarly accomplishments or faithful teaching service did not have the headline appeal demanded by newspapers. By overemphasizing the importance of discovery in research and being unwilling to exchange some of that excitement for the calmer and slower maturing pleasures of teaching, we may not only have allowed our own instructional programs to deteriorate but also have encouraged indirectly a reduction of teaching effort in other fields. Thus, intellectual breadth was pushed aside by the force of scientific progress.

What has been said in no way questions the desirability of continued progress or expanded research work in dental science. It does, however, suggest that an infinite expansion of such activities is not desirable unless care is taken to guard against weaknesses of the sort which have been suggested. If we admit that our approach to professional and social needs has not been as broad as it might have been, then we should look towards improving the situation. A consideration of what can be done is worth attempting.

Dental research workers have responsibilities other than to themselves. Therefore, those of us in scientific research should remember that the opportunities for doing the work which we want to do have been provided by dentistry, and that we have a primary responsibility not to overindulge our own mental whims. We should keep in mind that although the particular enthusiasm of the moment may, if successfully pursued, solve all dental problems there is a much greater probability that it will not. Instead of the mental self-indulgence which some research workers confuse with intellectual freedom, more of us should use mental self-discipline to keep us working on other people's and, therefore, less interesting, ideas. Dentistry and science might benefit if less apparatus and more odd scraps of paper were used to take care of our own pet ideas. In other words, it is too easy for the research worker to use the mental freedom which is essential for research as an excuse for self-indulgence and a sort of intellectual irresponsibility of which no organization can be allowed too much for too long.

If we bear in mind the fundamental educational purpose of research, research workers should cease to live in a world apart. We should shape our publications so that, instead of being a form of scientific exhibitionism, they become a well-oriented introduction into new scientific realms for those who have not had the opportunity to comprehend what we have had a chance to learn. It seems that we are under the same obligation of good manners to make our activities interesting to dentistry, our professional host, as we are to do the same for our social hosts. Certainly we cannot expect the profession at large to continue to be interested in us if we are not considerate or polite enough to make our work understandable to it.

We should realize that the trappings of research, like much that is fascinating, are also dangerous. This is true not only because it is too easy for an investigator to become confused by the complexity of his own apparatus but also because too great an emphasis on technical gadgetry discourages

those who do not have apparatus from attempting research of any sort. Dr. Gregg of the Rockefeller Foundation spoke wisely when he said that the critical quality of research is generally inversely proportional to the complexity of the apparatus used. Good research, but perhaps not fashionable research, can still be done with the traditional toothpick and a piece of string. Ideas are still the heart of research. In other words, metaphors are still as important in research as are microscopes. As long as research workers lose themselves in their laboratories and fail to encourage the idea that research is an attitude of mind rather than a matter of equipment, they can expect to be accused of being more interested in wearing the robes of the magician than in really trying to cure the sick.

* * * * *

The suggestions which have been made are but some of many which could be made. They have been chosen because they indicate steps which should be taken to ensure the best possible "public relations" for dental research. They really represent nothing more than an appeal for a recognition of our own shortcoming and a fuller appreciation of activities outside our own field of scientific research. I hope that, while implying complete confidence in the value of the research work we have been doing, I have raised a warning against allowing ourselves to get so wrapped up in our own small problems that we lose sight of the real purpose of our work and cease to be considerate of our allies in it. From those who work in teaching institutions I would ask the greatest care that in interpreting their work to students and faculty they guard against discouraging any sort of intellectual activity simply because it does not employ the techniques of experimental science. We should always realize that it is the short term reproducible scientific experiment alone which makes science what it is. If scientific research is not serving as a stimulus to intellectual activity of all kinds, it is doing less than it should. If such a situation exists, it is the serious obligation of all in the research field to take to heart the thoughts of the more critical scientific and nonscientific philosophers of today.

In spite of the warnings I have offered, I am not a pessimist about the part dental research can play in our future intellectual and professional growth. To me it seems that dental scientists have a rare opportunity to present basic science and research in an outstandingly effective professional and social framework. Dentistry has a relatively small area of responsibility in the health field. It is small enough that the basic clinical problem and techniques can be comprehended and used by one who is primarily a laboratory research worker, and it is easy for such men to have had enough clinical experience not only to appreciate the physical difficulties but also the humane satisfactions of dental practice. They should be able to understand that there is a psychological completeness coming out of such work which, although of a different sort, is comparable to that which research workers seek in their pursuits. And, conversely, the capable clinician does not have such a large mental responsibility that it is impossible for him to find space in his mind for consideration of scientific problems. The scientifically minded clinician may never unearth a significant scientific fact but, with encouragement from those with better scientific opportunity, he will learn much particularly in the area of mental satisfaction. This overlap of interest should be bound together by a mutual appreciation of the other's mechanical artistry or ingenuity of mind and by a common interest in the humanitarian goals of their profession.

If these mutual appreciations can be fostered, and the responsibility of doing this rests principally with the research worker, for it is he who has

introduced new techniques and new standards of value, then it should be possible to offer an ideal for all in dentistry who can see visions; a vision of a professional group permeated with the conviction that their profession offers a unique opportunity for all who will partake to enjoy a blending of the satisfactions of scientific inquiry, technical artistry, and humanitarian service. That goal is worth the best efforts of all of us.

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III. First Session: Friday Afternoon March 24; Abstracts 1-10

1. Human Mouth Antibiotics. *C. A. Scrivener, H. I. Myers, N. A. Moore, and B. W. Warner, School of Dentistry, University of Kansas City, Kansas City, Mo.* Two salivary pools were collected, one from caries-active and one from caries-resistant persons. Bacteriological Petri dish culture plates were prepared using various culture media. These media were selected to provide at least one type that would grow each major group of aerobic bacteria found in the mouth. The plates were inoculated with serial dilutions of the immune and susceptible pools and were incubated and examined at 24, 48, and 72 hours. As colonies started appearing, each that appeared different in any discernible way was transferred to the preferred media and subsequently isolated in pure culture. Thirty pure cultures were thus established; 17 from susceptible and 13 from the resistant salivas. Each of these pure cultures were tested for antibiosis against 3 strains of *L. acidophilus*. In some instances no promising antibiotic action was noted and these bacteria were discarded. A total of 14 pure cultures showing some antibiosis against *L. acidophilus* were tested farther. Each of these 14 oral test organisms were tested for antibiotic action against each other. The degree of antibiosis and number of organisms inhibited varied greatly. Not only is it seen that antibiotic bacteria can be influential but bacteria, possessing this property, exist in the oral cavity and they probably produce different antibiotic substances.

2. Effect of Oral Antibiotics on Tooth Fragment Weight Change. *C. A. Scrivener, H. I. Myers, N. A. Moore, and B. W. Warner, School of Dentistry, University of Kansas City, Kansas City, Mo.* The tooth weight gain or loss procedure was essentially the same as that used by Schantz and Scrivener except, instead of weighing samples from the same 12 bottles at intervals, the fragments from a different set of 12 bottles were used for each weighing. Aseptic technic was used throughout the experiment. Fourteen pure cultures of bacteria having antibiotic properties against *L. acidophilus* were isolated from susceptible and resistant saliva pools. These various bacteria were added singly to mixtures of *L. acidophilus*, broth, and tooth fragment series. The weight loss and gain of the fragments were recorded at 4, 24, 48, and 72 hours. Of the entire group tested, an organism isolated from resistant saliva was outstanding in its action. There was a definite weight gain at 4 hours that persisted until the termination of the experiment at 72 hours. When this organism was tested with susceptible saliva, a weight gain was recorded at 4 hours. With the addition of sucrose a rapid loss at all incubatory periods resulted. As it is known that sucrose increases proteolysis the explanation here could have been disintegration of the tooth particles. Another promising bacteria isolated from susceptible saliva and believed to be from the *B. subtilis* group showed a weight gain at 4 hours in broth; but never was the loss at 24, 48, and 72 hours as great as the control. Evidently some factor inhibited this loss. The factor that stood out in the majority of bacteria tested was the fact that a characteristic weight gain was recorded at 4 hours.

3. Calcium Determination in Tooth Weight Gain and Loss Experiments. *C. A. Scrivener, H. I. Myers, N. A. Moore, and B. W. Warner, School of Den-*

tistry, University of Kansas City, Kansas City, Mo. It was shown that tooth fragments subjected to saliva or nutrient broth containing *L. acidophilus* lost weight. When bacteria having antibiotic properties were added to nutrient broth and *L. acidophilus,* the weight loss was altered. It was decided to analyze the media colorimetrically for calcium to see if any correlation between it and weight loss occurred. Graphs showing correlation between weight and Ca loss were constructed. Antibiotic bacteria from susceptible and resistant saliva were added to sucrose broth, *L. acidophilus,* and tooth fragments. In most instances (11 out of 14) the calcium loss was essentially the same as the control graph. Three bacteria, 1 from susceptible saliva and 2 from resistant saliva showed decreased decalification in comparison with the control. Most of the 11 antibiotic bacteria mentioned above showed a characteristic weight gain at 4 hours. Judging from these experiments it appears that the ideal antibiotc bacteria would tend not only to inhibit the *L. acidophilus* but prevent a lowering of the pH, weight loss, and decalcification. Since 1 bacteria from each of the resistant and susceptible pools showed the greatest tendency to inhibit *L. acidophilus,* it was decided to use a salivary media instead of the broth. The susceptible bacteria did not prevent the weight loss entirely but practically none of the loss was due to calcium, i.e., none apparently was escaping from the tooth fragments. The bacteria from the immune pool showed only an erratic weight gain, or a weight loss when sucrose was added.

4. Factors Influencing the Detection of Streptococci in Blood Culture Studies. *Leonard Robinson, Joseph P. Lazansky, and Vilma Johnson, Tufts College Dental School, Boston, Mass.* An in vitro study was undertaken to evaluate the influence of blood-broth ratios, and the absolute amount of blood present on the detection of streptococci in blood-broth mixtures. Specific streptococcal strains were recovered from the blood of 6 patients after tooth extraction; each of these strains was tested with blood obtained from the same patient at a later date. Three syringes of blood containing 20 c.c., 20 c.c., and 13 c.c. of blood were obtained at 1 time from each subject, and such volume of a 10^{-6} bacterial suspension added so that the initial bacterial concentration in the blood-bacteria mixtures was 10^{-7}. The mixtures were then distributed among flasks containing varying amounts of tryptic digest broth, with the following blood-bacteria-broth mixtures resulting: 20:100, 10:100, 10:200, 4:20, 4:20 enriched with citrate and saponin, and 5:100. After incubation at 37° C. for 24 hours, serial dilutions from 10^{-2} to 10^{-9} were prepared from each mixture and pour plates were made from each of the serial dilutions. The pour plates were incubated for 48 hours, and colony counts made at that time. Bacterial growth was greater in those blood-broth mixtures where the ratio of blood to broth was small. Beginning at the 10^{-4} dilution, bacterial colony counts with the mixtures having a ratio of 1:20 were somewhat higher than with those having a ratio of 1:10 and considerably higher than with those having a ratio of 1:5. When citrate and saponin were added to the broth, the growth-promoting qualities of a 4:20 blood-broth mixture were enhanced. With a favorable blood-broth ratio, an increase in the amount of blood present seemed to render the mixture less favorable for growth.

5. The Effect of Variations in Sampling and Culturing Methods Used for Detection of Bacteremia. *Frederick W. Kraus, Joseph P. Lazansky, and Sunya Gordon, Tufts College Dental School, Boston, Mass.* A survey of the literature reveals the composite incidence of streptococcemia following single extractions to be much lower (22/208) than following the extraction of 2 or more teeth (135/202). Our findings, based on 405 extraction cases, suggest that this difference may be due to the magnitude of the bacterial invasion and

the effectiveness of the methods used for detection, rather than to incidence. Large ratios of volume of drawn blood to volume of broth are unfavorable for the growth of nonhemolytic streptococci (10:50 ratio: no positives in 43 cases; 20:100 ratio; 8 per cent positives in 152 cases). Detectability increases with decreasing blood-broth ratio (10:100 shows 15 per cent positives in 191 cases; 5:100 shows 33 per cent positives in 84 cases). Increased blood dilution eliminates the difference between the incidence in single and multiple extractions (10:100 dilution: 4/72 in single vs. 24/119 in multiple extractions – chi square = 6.53; 5:100 dilution: 5/24 vs. 23/60 - chi square = 1.64). A similar effect can be achieved by the use of citrated saponified broth (28 per cent positives in 109 cases, 10/34 vs. 21/75 - chi square = < 1), whereby the blood-broth ratio seems to be less important. As a tentative explanation, both methods are thought to inhibit the antibacterial activity of the drawn blood and to raise its value as a nutrient. Detection can be further enhanced by dividing the blood sample into several aliquots cultured simultaneously by favorable methods (38 per cent positives in 102 cases, 8/24 vs. 31/78 - chi square = < 1). The advantage in culturing more than 1 aliquot may be explained by assuming an uneven distribution of viable bacteria in the blood.

6. Vitamins Needed for Growth of Some Oral Lactobacilli. *Stewart A. Koser and Barbara Jane Fisher, Walter G. Zoller Memorial Dental Clinic and the Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.* Oral lactobacilli from saliva or carious lesions were employed for this study; some were recently isolated, others from the laboratory stock collection. To determine the vitamin needs, a basal medium of acid-hydrolyzed casein, certain amino acids, glucose, acetate or citrate, and inorganic salts was used. In such a medium, without added vitamins, all of the lactobacilli failed to grow. The needs of each culture were determined by adding to the basal medium various combinations of the vitamins. Of 20 strains of oral lactobacilli studied, all require for growth nicotinic acid, pantothenic acid, and biotin; many need also pteroylglumatic acid, vitamin B_6 (added as pyridoxamine), or perhaps both; some need riboflavin, others thiamine. Growth through repeated transfers was obtained in the presence of the appropriate vitamins. Omission of any one of the needed vitamins prevents growth or results in greatly decreased amounts of growth and resultant metabolic products. Certain purines and pyrimidines often stimulate growth in the presence of needed vitamins but do not substitute for them. (To be published in *J. D. Res.*)

7. Frequency Distribution of Lactobacillus Types in the Salivas of Children. *Morrison Rogosa, Joyce A. Mitchell, and Robert J. Fitzgerald, National Institute of Dental Research, National Institutes of Health, Bethesda, Md.* Ninety-nine saliva samples, which were obtained from children in the first through the sixth grades, were plated on tomato juice agar (pH5), and 360 representative isolates of lactobacilli were replated until pure cultures were secured. All gram-positive, catalase-negative rods producing acidity from sugars were accepted as lactobacilli. Organisms producing gas (CO_2) were designated as heterofermentative as distinguished from the non-gas producing homofermentative lactobacilli. For further subdivision, fermentation tests were performed with 20 carbohydrates. Total acidities in milk, limits of temperature for growth, and the vitamin requirements of the organisms were determined. Based on these tests it was found that the frequency distribution of the total sample was: *L. casei*, 38.05 per cent; *L. acidophilus*, 10.57 per cent; *L. plantarum*, 2.54 per cent; new species (homofermentative), 2.33 per cent; miscellaneous unidentifiable (homofermentative), 0.85 per cent; *L. fermenti*, 22.41 per cent; *L. brevis*, type I, 5.07 per cent; *L. brevis*, type II, 4.86 per cent; new species

(heterofermentative), 10.78 per cent; and miscellaneous unidentifiable (heterofermentative), 2.54 per cent. Of this total sample, 45 per cent were heterofermentative and 55 per cent homofermentative types. In addition, 114 strains of lactobacilli were isolated from saliva samples plated on tomato juice agar containing one unit of penicillin per ml. The strains in this group comprised 85 per cent heterofermentative and 15 per cent homofermentative lactobacilli.

8. Types of Acidogenic Lactobacilli Isolated From Saliva. *Evelyn B. Tilden and Muriel Svec, Dental and Medical Schools, Northwestern University, Chicago, Ill.* A group of cultures of lactobacilli, most of them isolated from the salivas of orphanage children by the method of Davies, Slack, and Tilden (calcium phosphate plate) has been compared as to their (1) production of acid on glucose broth, (2) capacity to produce acid and gas on glucose agar (Snyder's medium), (3) action on milk, (4) action on carbohydrates, and finally, (5) their growth and acid production on a synthetic medium devised for microbiological assay of phenylalanine by means of *L. casei*. The proportion of strongly acidogenic cultures (final pH below 4.0 in glucose broth) was high, 74 per cent of the total, as would be expected from the method used. Eighteen lactobacilli of the gas-forming (heterofermentative) type were identified, 13 of these evidently being *L. fermenti*. Forty-five cultures were obtained which appear to be *L. casei*. The group of *L. casei* includes 7 cultures isolated from root canals in an earlier study. Sixteen strains were lactose-negative and have not yet been identified. The remaining 29 strains resembled *L. casei* in their acidogenic capacity and fermentation tests but failed to grow on the synthetic medium. None of the 101 cultures studied resembled *Lactobacillus acidophilus* as defined by Pederson or by Tittsler et al.

9. Metabolism of Oral Lactobacilli. *T. B. Coolidge, Walter G. Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* The metabolic transformations of glucose by several strains of oral lactobacilli and the change in glucose metabolism occurring with the change in fermentation described by Harrison have been studied. The strains of lactobacilli statistically more closely associated with caries are homofermentative and yield practically quantitatively 2 moles of lactic acid per mole of glucose. The remaining strains studied are heterofermentative yielding about 1 mole of lactic acid, 1 mole of carbon dioxide, and a fraction of 1 mole of ethanol per mole of glucose. The changes described by Harrison are accompanied by a change from the heterofermentative to the homofermentative type of metabolism.

10. Effect of Certain Hendecynoic Acids and Their Ammonium Salts on Acid Production in Saliva Containing Glucose. *J. C. Forbes, C. D. Cox, and J. D. Smith, Medical College of Virginia, Richmond, Va.* Studies carried out with 2-, 6-, 9-, and 10-hendecynoic acids (11 carbon fatty acids having a triple bond), as well as their ammonium salts, show that they exert a marked inhibitory action on the production of acid in saliva containing glucose. Acid production was measured both by the drop in pH and by the solution of calcium from bone and tricalcium phosphate. Studies with pure cultures of *Lactobacillus acidophilus*, using 9-hendecynoic acid, indicate that the main effect is on inhibition of growth of the aciduric organisms rather than on an enzyme system involved in acid production. (Published in *J. D. Res.* **29**: 583, 1950.)

IV. Second Session: Friday Evening, March 24; Abstracts 11-20

11. Reattachment of the Marginal Gingiva and Periodontal Membrane in Experimental Animals. *Irving Glickman and Joseph P. Lazansky, Tufts College Dental School, Boston, Mass.* Reattachment of the marginal gingiva

and periodontal membrane following the gingivectomy and "flap" operations. were compared histologically. In 10 different quadrants the following 2 operations were performed in each of 5 dogs with chronic marginal gingivitis: (1) gingivectomy entailing removal of the marginal gingiva, epithelial attachment, alveolar bone, and scarification of the cementum to a point ¼ inch below the cemento-enamel junction; (2) same as the first operation except that the marginal gingiva was separated from the tooth to permit the other procedures and then retained in position with sutures. Microscopic examination of the experimental areas one month after operation revealed reattachment of gingival epithelium, restoration of the level of the marginal gingiva, formation of new cementum, reattachment of periodontal membrane fibers; bone apposition were essentially the same regardless of whether the marginal gingiva was resected or allowed to remain in position. Healing in the periodontal area was compared with that following the surgical removal of a spherical section of the cortical plate of alveolar bone which in some cases was accompanied by the resection of the overlying oral mucous membrane and in others covered by a flap sutured in position. In general, the healing of such lesions was comparable to that in the periodontal area. The retention of a mucous membrane flap over the zone of operation diminished inflammatory involvement which retarded the healing process.

12. Studies in Periodontosis. *Maxwell Karshan, Benjamin Tenenbaum, and Daniel E. Ziskin, School of Dental and Oral Surgery, and College of Physicians and Surgeons, Columbia University, New York, N. Y.* One hundred thirty-six cases from 14 to 47 years of age in various stages of periodontosis were studied. The investigation included comprehensive dental examination, blood chemistry, blood counts, neutral 17-ketosteroids in urine, Kline test, basal metabolism, dietary record, and physical examination. Increased blood values were found for (1) glucose in 15.7 per cent of the periodontosis cases, (2) globulin in 26.3 per cent, (3) alkaline phosphatase in 10.8 per cent, (4) erythrocyte sedimentation rate in 42.2 per cent, (5) erythrocyte count in 15.2 per cent, (6) leucocyte count in 12.3 per cent, and (7) neutrophiles in 12.3 per cent, as compared with 1.8 per cent for glucose, 3.2 per cent for globulin, 0 per cent for alkaline phosphatase, 11.5 per cent for erythrocyte sedimentation rate, 3.1 per cent for erythrocyte count, and 0 per cent for both leucocyte count and neutrophiles in a control group of 51 cases free from periodontal disease. Decreased values were found for (1) vitamin C in 33.1 per cent of the periodontosis cases, (2) albumin in 13.5 per cent, (3) calcium ion in 12.9 per cent, (4) erythrocyte count in 12.4 per cent, as compared with 15 per cent for vitamin C, 3.2 per cent for albumin, 5.7 per cent for calcium ions, and 3.1 per cent for erythrocyte sedimentation rate in the control group. No appreciable differences were found for total calcium, inorganic phosphate, total protein, vitamin A, carotene, sugar, nonprotein nitrogen, and hemoglobin. The Kline test was negative in all cases and the basal metabolic rate was outside of normal limits in 16 per cent of the cases. This did not differ appreciably from the control group. The food eaten during a 1-week period was recorded, and from these data it was calculated that the intake was low in the following percentage of periodontosis cases for (1) calories in 48 per cent, (2) protein in 24 per cent, (3) calcium in 70 per cent, (4) phosphorus in 58 per cent, (5) iron in 45 per cent, (6) thiamin in 21 per cent, (7) riboflavin in 50 per cent, and (8) niacin in 41 per cent. Twenty-two control cases gave essentially the same dietary results. The average value for 17-ketosteroids in urine for 22 females between the ages of 14 to 47 years in the periodontosis group was 9.2 mg. per 24 hours' specimen as compared with 11.0 mg. in a control group of 17 females between

the ages of 20 and 57 years. Physical examinations revealed a large number of unrelated conditions; no consistent pattern has been found.

13. The Incidence of Penicillin-Fast Lactobacilli in the Salivas of Children Using a Penicillin Tooth Powder. *Robert J. Fitzgerald, National Institute of Dental Research, National Institutes of Health, Helmut A. Zander, Tufts College Dental School, Boston, Mass., and Harold V. Jordan, National Institute of Dental Research, National Institutes of Health, Bethesda, Md.* Salivary lactobacillus counts on tomato juice agar with and without added penicillin were performed on a control group of children and an experimental group using a penicillin-containing dentifrice to determine the incidence of penicillin-fast lactobacilli. In June, 1949, using tomato-juice agar plates containing 1.0, 10, and 50 units of penicillin per ml. of medium, the incidence of positive plates for the lowest penicillin concentration was 14 per cent of 129 control children and 22 per cent of 181 cases in the experimental group. At this time the dentifrice had been in use for 1 year and a half. None of the plates containing higher concentrations of penicillin gave positive cultures for lactobacilli. Six months later the incidence of positive plates containing 1 unit penicillin per ml. of medium was 24 per cent and 27 per cent, respectively, for the control and experimental groups. Under the experimental conditions employed, these differences could not be considered significant. (Published in *J. A. D. A.* **41**: 62, 1950.)

14. Protein Deprivation and the Periodontal Structures of the Albino Rat. *Irving Glickman and T. N. Chawla, Tufts College Dental School, Boston, Mass.* An experiment was conducted to evaluate the changes in the periodontal tissues associated with protein deprivation. Eighty male and female littermate albino rats, Wistar strain, 25 days old and weighing 45 to 60 grams, were used as experimental animals. Forty rats were fed on a synthetic diet complete in all respects except that the protein content of the diet was reduced to 2 per cent. The remaining 40 rats were used as controls and fed a similar diet with the protein content raised to 22 per cent by the addition of casein. The diets in both groups were isocaloric and water was allowed ad libitum. An equal number of animals was sacrificed from each group at the following time intervals during the 8-week experimental period: 2, 4, 6, and 8 weeks. At the time of sacrifice the mandible, maxilla, vertebra and femur were removed, x-rayed, and prepared for histologic study. There were no clinically detectable periodontal changes in either group of animals. Microscopic examination of the periodontal structures revealed the following features in the protein-deficient animals: osteoporosis of the alveolar bone characterized by thinning of the trabeculae and diminished apposition of osteoid matrix; a tendency toward necrotizing gingival changes in the presence of local irritants; no tendency toward pocket formation; and diffuse edematous involvement of the periodontal membrane.

15. The Connective Tissue Fibers of the Marginal Gingiva. *David A. Hagerman and Sumter S. Arnim, School of Dentistry, University of Texas, Houston, Texas.* Studies of serial sections of gingivae of human, monkey, and rat jaws reveal a compact band of connective tissue fibers encircling the teeth. This circumferential band is seen in normal tissues of the three species. This presentation calls attention to this band of fibers. It is seen in serial cross sections of the marginal gingiva made in a horizontal plane. Search of the literature reveals no definite description of this particular group of fibers. The term "ligamentum dentis" has been used to refer to fibers in this general area but was not applied to this particular group. The circumferential band is seen in healthy submucosa underlying the epithelium of the free margin of the gum. There is a marked difference in the band in health and disease. The healthy

band is composed of many closely interwoven fibers forming a compact, well-differentiated group. In diseased gingivae, the fibers are few in number, separated widely by cellular and serous exudate, and are difficult to see. The arrangement of fibers in the circular band may aid in maintaining close apposition of the marginal gingiva to the tooth. The band was recognized as a separate entity because many serial cross sections of normal tissue cut in a horizontal plane were studied. Now that its presence is known it may be recognized in cross section in many mesiodisto-axial photomicrographs published by others.

16. The Effect of Radioactive Phosphorus Upon the Development of the Embryonic Tooth and Supporting Structures. *M. S. Burstone, Washington University School of Dentistry, St. Louis, Mo.* Pregnant C57 black mice were injected subcutaneously with radioactive phosphorus in dosages from 5 to 17 microcuries per gram of body weight. The offspring were sacrificed at varying time intervals, and serial sections of the jaws and mandibular joint were studied histologically. With dosages of 10 to 17 microcuries per gram of body weight (of pregnant mouse), marked disturbances of osteogenesis and odontogenesis are produced in the offspring. In some areas there is a cessation of histodifferentiation of ameloblasts and odontoblasts. Some ameloblasts appear as squamous type or low cuboidal epithelium and are without function. The odontoblasts appear as spindle cells with cytoplasmic processes. These cells are associated with osteodentin formation. The development of the third molar tooth is completely inhibited. The greatest odontoblastic and ameloblastic distortion is present toward the root portion of the crown where these cells have most recently differentiated. Thus, the radiosensitivity and radioresistance of the odontoblasts and ameloblasts follow a similar pattern except that the odontoblasts are far more radiosensitive. Alterations in the condyle are suggestive of a premature ageing process. Marrow aplasia, premature subcartilage linkage by immature fibrous bone, and decreased osteoblastic activity are characteristic changes. Dosages required to produce the changes described are less than what would be required to produce similar changes by injection of immature mice. This suggests that either the embryonic tooth and supporting structures exhibit a greater radiosensitivity or that because of the rapid growth of embryonic tissues, the uptake of P32 is such as to concentrate this radioactive material in the tissue, thus producing a greater intensity of radiation.

17. Retention of Foodstuffs in Different Mouths. *B. G. Bibby and Eugene Chen, Eastman Dental Dispensary, Rochester, N. Y.* Although in the hands of trained persons tooth brushing has provided a satisfactory method for recovering the test foodstuffs from the teeth, it is not suitable for use in ordinary patients. Repeated tests showed that careful use of an atomizer gave parallel results to skillful tooth brushing, and food retention studies became possible on untrained persons. Wide variations on the retention of the same foodstuffs were found in different individuals. While these differences were determined in a large measure by the dental condition of the patients, being highest where gingival recession and many tooth spaces were present, the results were not parallel for all foodstuffs. Mouths which showed up to three times retention of other mouths with one foodstuff sometimes gave equal retention with other foods. These differences are not related directly to the total amount of retention of different foods and may be influenced by the effects of fat on the cleaning mechanisms of different mouths.

18. Retention and Decalcification Potentialities of Common Foodstuffs. *Eugene Chen and B. G. Bibby, Eastman Dental Dispensary, Rochester, N. Y.* Studies have been continued on food retention (determined by carbo-

hydrate analysis of hydrolysed tooth washings) and decalcification potentials (derived by multiplying mg. of food retained and acid formed on incubation with saliva) of different foodstuffs. As a further test of the accuracy of the test procedures, figures were obtained in the tests in which foods were expectorated instead of being swallowed. These showed a maximum of 5 per cent error in the recovery figures, and indicates a surprisingly high degree of accuracy in the test methods. In an effort to determine what factors are responsible for the differences of the retention of foodstuffs on the teeth and differences in their acid production, experimental "laboratory cakes" were made of varying quantities of known ingredients. "Laboratory cakes" baked with lard gave lower retention figures and lower acid formations than those that were baked with peanut oil. Some types of fats or proteins seem to promote higher acid production in food-saliva mixtures. The addition of baking powder to "laboratory cakes" lowered food retention but increased acid production. The addition of sodium chloride gave lower food retention and acid production figures.

19. Possible Ectodermal Origin of the Odontoblast. *James K. Avery, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Development studies of the salamander, *Ambystoma maculatum,* have revealed the migration of neural crest (N.C.) cells into the region of the future mandible and maxilla. These N.C. cells appear intermingled with the mesodermal cells along the outer border of the stomodeal collar, the future oral ectoderm. The N.C. cells appear to be incorporated in the pulpal tissue of the developing teeth, forming processes which pass into the tubular dentin. No other cells within the pulp appear histologically to develop into odontoblasts. Alkaline phosphatase appears to be localized in the neural crest cells under the epithelium of the future oral ectoderm, as well as the oral ectoderm itself.

20. Water Consumption in Relation to Environmental Temperature and Its Significance in the Fluoridization of Water Supplies. *Margaret Matuschak Levin and Gerald J. Cox, School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* The amount of water drunk by two children and one adult was measured in relation to the mean daily temperature. The objective was to establish a basis for the seasonal variation of the fluoridization of community water supplies pending determination of the optimum amount of flourine for the formation of caries-resistant teeth with a minimum of mottled enamel. For the first 152 days of life for a girl, 139 observations were obtained beginning with birth on May 13, 1949. The data are summarized by the *interpolation* equation,

$$W = 3.313 + 0.253T + 0.0098T^2$$

in which W = ounces of water per 10 pounds' body weight and T = mean daily temperature measured from 70° F. in the range 56 to 84° F. Water consumption by a boy, age 21 ½ months at the start from 56 observations is shown by

$$W = 3.779 + 0.170T + 0.0063T^2$$

The data for the mother of the above children, aged 33, for 60 observations are summarized by

$$W = 3.640 + 0.0864T + 0.00144T^2$$

V. Third Session: Saturday Morning, March 25; Abstracts 21-28

21. The Accessibility of the Organic Dentinal Matrix. *George W. Burnett and Henry W. Scherp, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.* As part of a study of the role of proteolytic micro-

organisms in dental caries, an investigation has been made of the accessibility of the protein of dentin to chemical and microbiological attack. Accessible protein was detected by coupling it, presumably through its tyrosine and histidine residues, with diazonium compounds to form colored azoprotein derivatives, which were dissolved in alkali and measured colorimetrically. The protein was essentially inaccessible until the dentin was partially or completely decalcified, when the amount of azoprotein formed was directly proportional to the weight loss. (Naturally carious dentin formed only 50 per cent as much azoprotein per mg. of tissue.) Concurrently, the organic matrix became susceptible to lysis by proteolytic enzymes from animal and bacterial sources. Actinomycetes, enterococci, and lactobacilli isolated from deep dentinal caries acted upon dentin while growing in thioglycolate or glucose broth to release the organic matrix for coupling and proteolysis. After exposure to proteolytic bacteria, isolated from superficial dentinal caries, dentin reacted with diazonium compounds, but the color was not proportional to the weight loss. Azoprotein prepared from dentin decalcified by acids or by microorganisms was decolorized and lysed by the actinomycetes and proteolytic bacteria. The results indicate that the organic material of dentin becomes accessible to chemical and microbiological attack after its linkage to the inorganic matrix is broken, as by acid. Changes other than decalcification occurred, however, even at the low acidity of pH 3, for small amounts of nitrogenous substances were found in the decalcifying solution.

22. The Estimation of Some of the Amino Acids in Dentin Protein. *W. C. Hess, F. L. Losee, and B. Neidig, Georgetown Medical and Dental School and the Navy Dental School, Washington, D. C. and Bethesda, Md.* The dentin from noncarious first molars was demineralized by dialysis against 2 per cent acetic acid followed by 2 per cent hydrochloric acid. The protein, remaining in the dialysis cell, was dried and used for the study. The yield was 8.11 per cent of protein containing 15.56 per cent nitrogen based upon a desiccator-dried sample corrected for ash. The amino acids were determined by colorimetric and microbiologic methods. The colorimetric analyses were performed in our laboratories while the microbiologic assays were carried out on our hydrolysates under the direction of the microbiological laboratory of the National Institutes of Health. The following values, expressed as per cent of the weight of the desiccator dried sample, were obtained by the colorimetric methods, cystine 0.08, methionine 0.49, phenylalanine 1.92, arginine 7.40, histidine 0.66, hydroxyproline 13.26, and by microbiologic assay arginine 7.29, histidine 0.82, and lysine 3.43, and by paper partition chromatography lysine 3.30. The results of these determinations will be discussed in relation to the concept that dentin protein is collogen in nature.

23. Absorption of Enzyme Inhibitors by Proteins. *L. S. Fosdick and W. E. Ludwick, Dental School, Northwestern University, Chicago, and Dental Department U. S. Naval Training Center, Great Lakes, Ill.* It was found that when enzyme inhibitors are incorporated in a dentifrice, and the dentifrice used morning and night, reductions in caries activity rarely results. Of seven inhibitors that were tested in this manner, only penicillin was active, and tyrothrycin was partially active. It was thought that perhaps penicillin would absorb into the dental plaque proteins and hence be present when food is ingested. For this reason the absorption of the inhibitor was studied on gelatin and casein. It was found that most of the inhibitors would be partially absorbent onto gelatin, but of the inhibitors tried, only penicillin would absorb on casein. It is suggested that this may be a test for the effectiveness of enzyme inhibitors when incorporated into dentifrices.

24. A Histochemical Study of the Calcification of the Mandible. *Gerrit Bevelander and P. L. Johnson, College of Dentistry, New York University, New York, N. Y.* The calcification of the developing mandible of the pig has been investigated by histochemical methods with special reference to time relations and intensity of the following: (1) alkaline phosphatase, (2) glycogen, (3) mucopolysaccarides, and (4) mineralization. Utilizing the Altmann-Gersh freeze-dry method of fixation, we have observed in the material treated in this manner that alkaline phosphatase is present in osteogenic tissue and cells, in osteoblasts, in the fibers of precalcified bone, and in osteocytes. Glycogen and mucopolysaccharides are present in osteogenic cells and fibers also. Glycogen, however, is present in osteoblasts and osteocytes of fairly well-developed bone spicules. Mucopolysaccharides appear in the fibers and cells of osteogenic tissues, but is only faintly visible in young bone spicules. As mineralization occurs, there appears to be disappearance of phosphatase from the matrix and a corresponding increase in the amount of the polysaccharide; glycogen in the osteocytes remains meanwhile fairly constant. Our studies indicate that there is a definite correlation between the presence of phosphatase, glycogen, and mucopolysaccharides in connection with the development of the organic and inorganic constituents of a developing membrane bone.

25. Rates of Calcium Exchange in Dental Hard Tissues. *Harold C. Hodge and Elizabeth E. Underwood, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Powdered dentin and enamel samples were stirred in calcium chloride solutions containing radioactive calcium, C_{45}, for increasing time periods up to several days. The percentages of the radioactive calcium taken from the solution by the dentin and enamel increased apparently toward a maximal value. The rates of these exchange reactions have been studied and compared to the rates for powdered bone and for a synthetic hydroxyl apatite.

26. Oral Manifestations of Ionizing Irradiation. I. Lesions in the Oral Mucosa, Tongues, Developing Teeth, Salivary Glands, and Contiguous Tissues of Irradiated Swine. *James A. English and John L. Tullis, Naval Medical Research Institute, Bethesda, Md.* The material used in this investigation was taken at autopsy from 39 swine selected from a total of 128 which were exposed to total body 2000 kv. x-radiation in doses varying from 200 to 800 roentgens. Typical gross lesions which occurred in the first 5 days include ecchymotic and petechial hemorrhage of the oral mucosa (the peritonsillar area, sides of the tongue, under surface of tongue, soft palate, buccal and labial mucosa, gingiva, and hard palate). After the first week, necrosis and ulcerations occurred in the listed areas, particularly on the tip and sides of the tongue, the labia, and the hard palate. The latter areas are subject to traumatic injury, which is not well tolerated by irradiated tissue. Sections of regional lymph nodes show loss of lymphocytes, particularly in the nodules, and hemorrhage into the red pulp. Sections of the mandible through developing first and second molars revealed pronounced hemorrhagic tendencies of the entire crypt, the capsule, stellate reticulum, or developing pulp. Long-term effects on developing teeth will be determined from survivors of the experiment. Changes which were found in salivary glands, consisting of damage to duct epithelium, variation in zymogen granules, and reduction in the number of acinar nuclei, are probably not only due to primary x-ray damage but also due to secondary effects of enzymatic action and a possible role in the alarm reaction.

27. The Effect of Irradiation on the Developing Dental System of the Syrian Hamster. A Roentgenographic Study. *Karl W. Bruce, D.D.S., Mayo Foundation, Graduate School, University of Minnesota, Rochester, Minn.*

In this preliminary study an attempt was made to develop technics and materials for use in determining the roentgenologic changes of the skull, jaws, and dental system of the Syrian hamster subjected to radon irradiation and to carry out preliminary observations on the occurrence of such changes. The heads and jaws of 6 series of hamsters, each in various stages of development, were exposed respectively at 5, 10, 20, 30, 40, and 70 days of age. Each series consisted of 5 treated animals and a comparable number of untreated control animals. Each treated animal received 3 hours' irradiation time to the jaws, with variable doses. Treated and untreated animals were killed at 100 days of age, and a comparison was made by study of roentgenograms of the heads. The obvious gross effects of irradiation appear to be graying of the hair of the exposed parts and retardation of the development of the skull. Dental growth and development of the treated animals were impeded. This impedance was particularly pronounced in irradiated animals of the younger age groups as determined by measurement. Gross dental changes noted in the roentgenograms were anodontia, dwarfing of the teeth, and dwarfing of the roots. These gross changes were confined entirely to the series of animals that were irradiated at 5 and 10 days of age. Osseous growth and development seemed to be curtailed by irradiation. This curtailment was especially true in the series of immature animals. Slight or no effect of irradiation with respect to osseous underdevelopment occurred as the maturity of the animal increased.

28. A Demonstration of the Importance of Bicarbonate as a Salivary Buffer. *S. Wah Leung. School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Human stimulated mixed saliva and dog parotid and submaxillary saliva obtained by pilocarpine stimulation were equilibrated at 37° C. with gas mixtures containing carbon dioxide at tensions varying from 0 to 150 mm. Hg. The equilibrated samples were then analyzed for their CO_2 content and pH. The CO_2 dissociation curves thus obtained were used to calculate the total buffer value (B) of the saliva, as well as the values of the constituent buffers, especially bicarbonate, phosphate, and protein. The results indicate that dog parotid and submaxillary salivas owe their buffering capacity almost entirely to the bicarbonates. Human saliva shows rather wide differences in the total buffering values. In all cases the bicarbonates were found to be responsible for from 80 to 90 per cent of the total buffering capacity. Phosphates and proteins are thought to account for the remaining 10 to 20 per cent.

VI. Fourth Session: Saturday Afternoon, March 25; Abstracts 29-40

29. Further Studies on the Effect of Chlorophyll on Experimental Dental Caries. *William G. Shafer and John W. Hein, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Previous studies have shown a reduction in experimental hamster caries by the use of sodium copper chlorophyllin. In order to investigate this more thoroughly, and also in an attempt to explain the sex difference in response, 175 hamsters were placed in the following groups with sex and littermate distribution: I, control ♂; II, control ♀; III, castrated ♂ + chlorophyll; IV, castrated ♂; V, ovariectomized ♀ + chlorophyll; VI, ovariectomized ♀; VII, control ♂ + chlorophyll; VIII, control ♀ + chlorophyll; IX, control ♂ + 0.2 per cent chlorophyll in diet; X, control ♀ + 0.2 per cent chlorophyll in diet. Except in groups IX and X, the chlorophyll was administered at a level of 0.2 per cent in the drinking water. All groups received a cariogenic diet (CPD) for 165 days. Resulting percentage reductions in mean caries scores, compared to Group I, were: Group III, 80 per cent, Group IV, 46 per cent, Group VII, 45 per cent, Group IX, 78 per cent; compared to Group II: Group V, 9 per cent increase, Group VI, 24

per cent, Group VIII, 66 per cent, and Group X, 83 per cent. Three separate lactobacillus counts of all animals showed no significant changes due to sodium copper chlorophyllin. The material was also tested for a systemic influence on caries in 25 animals by administering during the formation of the teeth a 0.2 per cent chlorophyll drinking solution to the mothers of these animals from mating to 13 days after birth. The CPD was initiated at mating. Distilled water and CPD were given to the mothers of 26 control animals. At sacrifice, no differences were observed in the mean caries scores of control and experimental groups.

30. The Effect of Penicillin Dentifrices on Hamster Caries. *Frederic R. Shiere and Helmut A. Zander, Tufts College Dental School, Boston, Mass.* One hundred twenty-five hamsters were divided according to littermates and males and females into 6 groups. Immediately after weaning, they received a caries-producing diet. Group 1 served as a control group (no brushing). Group 2 had their teeth brushed daily with a tooth powder without penicillin; Group 3 with a powder containing 62.5 units of penicillin per Gm. of powder; Group 4 with a powder containing 125 units per Gm.; Group 5 the same powder but containing 250 units per Gm., and Group 6 the same powder but containing 500 units per Gm. The results were assembled in charts and graphs showing that there is, as far as hamsters are concerned, a critical effective level of penicillin beyond which there is little further beneficial result.

31. The Effect of a Penicillin Dentrifice on Caries Incidence on School Children. *Helmut A. Zander, Tufts College Dental School, Boston, Mass.* Three hundred fifty-two elementary school children were used for this study. All had full-mouth x-rays and bitewing x-rays and complete mouth examinations at the beginning. They were then divided into equal groups according to previous caries experience, age, racial background, and economic level. The control group had 150 children, the group receiving the penicillin dentifrice, 202 children. The dentifrices were identical except that one contained 500 units of penicillin per Gm. of powder. These children brushed their teeth once daily during school periods under supervision. They were re-examined at the end of 1 year and at the end of 2 years. Again x-rays were available for these 2 examinations. The results showing the decrease in caries incidence for each of the 2 years are assembled in graphs and charts. (Published in *J.A.D.A.* 40: 569, 1950.)

32. Analysis of the Health of the Children on the Penicillin Dentifrice Study. *Margaret Drolette, Harvard School of Public Health and H. A. Zander, Tufts College Dental School, Boston, Mass.* Careful records of absences from school were kept 12 weeks before the children received the dentifrice. These same records were continued for the 2 years of the study. A statistical analysis was made of these data and it was found that there was considerable difference between the group using the penicillin dentifrice and the control group before the dentifrice was introduced. Health of children is such a highly variable phenomenon that the effect of some factor which would produce a small effect cannot be demonstrated.

33. Penicillin Fastness on Streptococci and Staphylococci in the Throats of the Children From the Penicillin Dentifrice Study. *H. E. Lind, Sias Laboratories, Brooks Hospital, Brookline, Mass., and Helmut A. Zander, Tufts College Dental School, Boston, Mass.* Throat smears were obtained from 154 children using a control dentifrice and from 190 children using the same dentifrice but containing 500 units of penicillin per Gm. These smears were placed on blood agar plates in order to produce isolated colonies.

Selected colonies were picked into brain heart infusion broth, grown for 18 to 24 hours, and restreaked onto blood agar to insure purity. Colonies were then picked for penicillin sensitivity tests. The serial tube dilution method was used starting with 10 units per c.c. followed by twofold dilution down to 0.0011 unit per c.c. The tube containing the least amount of penicillin, showing complete inhibition, was selected as that amount to which the organism was sensitive. If one would call arbitrarily streptococci and stapylococci penicillin fast if the organism grew in the presence of 1.25 units of penicillin per c.c. or more, there were 6 penicillin-fast streptococci and staphylococci in both the control and the penicillin groups. In other words, the penicillin tooth powder did not affect the penicillin sensitivity of streptococci and staphylococci from the throats of the children using this powder.

34. The Effect of Partly Synthetic Diets on the Incidence of Dental Caries in the Hamster. *Frank J. Orland, Elizabeth S. Hemmens, and R. W. Harrison, Walter G. Zoller Memorial Dental Clinic and the Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.* Presupposing that certain oral bacteria are important in the development of dental caries, this investigation concerned itself with altering the nutritional substrate available to bacteria in the hamster's oral cavity. Partly synthetic, experimental diets containing 11 vitamins in definite amounts, with 1 or 2 of these vitamins being omitted from each test diet, were fed to 682 hamsters distributed into 18 different dietary groups for a 100-day period. During this time, the animals were provided with the missing vitamins by intraperitoneal injection for their own nutritional needs. As a plausible determinant of the degree to which certain oral bacteria were retarded or accelerated in their growth and metabolic activities, the extent of the carious lesions in the hamsters' molar teeth were evaluated. The following tendencies were observed: (1) When vitamin K was absent in the diet, hamsters showed the greatest amount of dental caries. (2) The animals fed a diet deficient in choline chloride developed about twice as much caries as animals in the control groups. (3) With the omission of nicotinic acid, the amount of caries was reduced to about one-half that of the control animals. (4) Animals receiving neither nicotinic acid nor inositol showed a higher degree of dental caries than the control animals. (5) Absence of vitamin E did not seem to prevent lesions from occurring, but apparently retarded their progress. (6) Substitution of dextrin for sucrose caused a 50 per cent decrease in molar destruction by dental caries.

35. An Influence on Dental Caries Incidence Produced in Rats by Tube-Feeding. *Owen W. Kite, James H. Shaw, and Reidar F. Sognnaes, School of Dental Medicine, Harvard University, Boston, Mass.* Rats of a caries-susceptible strain and of a suitable pre-experimental background, voluntarily ate, or were entirely tube-fed, a caries-producing diet for 16 to 25 weeks. Groups of intact and desalivated rats consumed the diet ad libitum while groups of intact and desalivated littermates were tube-fed comparable amounts. None of 13 intact rats which were tube-fed had tooth decay, while all but one of 13 controls had caries. None of 3 desalivated tube-fed rats had tooth decay, while all 4 desalivated controls had an extremely high caries incidence. These differences between control and experimental groups for intact and desalivated rats were highly significant. Critical ratios of the differences ranged from 5.7 to 26.6. The results of this preliminary experiment suggest that, with all other factors being controlled and equal, tooth decay is reduced in caries-susceptible rats when the direct effects of food in the oral cavity are eliminated. Preliminary histologic examination showed that the molar teeth of both groups were surrounded by a great abundance of microorganisms. In the controls, they

were in direct contact with the tooth surfaces; in tube-fed rats, they were separated from the enamel surface by a thick organic covering (possibly, remnants of Nasymth's membrane) and by foreign matter which was firmly impacted in the fissures. Further investigation is needed to determine the significance of the substances which separate the microorganisms from the tooth surfaces.

36. Association of Carious Lesions in the Proximal Surfaces of Human Teeth. *Gerald J. Cox and Jessica Witteman, School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* Dental records, supplied by the Dental Research Division, School of Medicine and Dentistry, University of Rochester (*J. D. Res.* 28: 648, 1949), were examined for the frequency of the joint occurrence of carious lesions in all possible pairs of abutting, bilateral, and heterolateral proximal surfaces in the permanent teeth of 457 14-year-old boys. Significant assocation was found in most of the abutting and bilateral pairs of surfaces but there was no significant association in the heterolateral pairs. Association in the abutting surfaces is ascribed to the dominance of the similar environment in promotion of decay; in the bilateral surfaces, to the similarity of conditions at the time of the formation of the teeth. Absence of association of decay in the heterolateral surface seems to be because of lack of the same environment, a difference in the time of formation of the teeth and a difference in the effectiveness of the factors which initiate decay. Asymmetry of decay in abutting and bilateral surfaces is ascribed to randomness of the initiation of the carious process.

37. Experimental Artificial Production of Dental Caries in Vitro. *Russell A. Hugill and Harold K. Box, Faculty of Dentistry, University of Toronto, Toronto, Ontario.* The purpose of this project has been to artificially produce a lesion in vitro in human teeth which microscopically manifests many of the characteristics of the pathological dental caries lesion in vivo, and in an experimental environment of controlled or inhibited bacterial activity. Bacteria and their metabolic products are generally accepted as the principal causative agents of the dental caries process. Subsequently, an experimental attempt was made to artificially imitate to some degree the actual nature of the destructive products of the bacteria in an attempt to reproduce an artificial dental caries lesion in vitro. First, the effects of various acids, both organic and inorganic, were studied. Results showed that decalcification caused by the acids in enamel and dentin does not fill the total requisites for the chemical activity of the caries process because some of the characteristics of the microscopical picture of caries were entirely absent. Second, the action of proteolytic enzymes, namely, pepsin, papain, trypsin, upon enamel and dentin were studied. Effects were produced which were different from and uncharacteristic of acid decalcification, but in fact were changes similar to the microscopical appearance of the pathologic lesion. Third, the acid and the enzyme experiments were followed by an experiment designed to combine proteolytic enzyme activity and acid decalcification simultaneously in one solution by subjecting teeth to solutions containing an acid and a proteolytic enzyme or enzymes. The results were extremely significant since a lesion was produced by these chemicals almost to microscopical exactness to the true pathological lesion. These results indicate that a controlled experimental caries lesion can be produced without bacterial activity to simulate the dental pathologic lesion.

38. The Effect of Various Dentifrices on Lactobacilli Counts and Caries Activity as Determined by the Snyder Test. *W. E. Ludwick and L. S. Fosdick, Dental Department, U. S. Naval Training Center, Great Lakes, Ill., and the Dental School, Northwestern University, Chicago, Ill.* The effect of

15 dentifrices on the lactobacillus counts and caries activity as determined by the Snyder test was made on 1,080 young Naval recruits. The subjects consisted of 18 groups of about 60 men each. The laboratory tests were performed at 3 consecutive times within a week after which 15 of the groups were supplied with dentifrices while the other 3 groups were used as controls. Those in the test groups were instructed in the use of a toothbrush and were directed to brush their teeth in the morning upon arising and in the evening before retiring. The 3 control groups continued their usual habits. The controls used the dentifrice of their choice, if any, and in the main brushed their teeth twice a day. The individuals were kept on this routine for from 5 to 8 weeks, and during the final week, 3 more lactobacillus counts and Snyder tests were performed. All of the test dentrifrices used contained an antibiotic, an enzyme inhibitor, or an active or potential base, and consisted of 13 tooth pastes and 2 tooth powders. In only 1 case, penicillin powder, was there a precipitous decrease in both lactobacillus count and in caries activity as determined by the Snyder test. All of the other groups showed sporadic fluctuations, and 1 group, those using tyrothrycin, showed some questionable benefit.

39. Histology of Dental Caries in the Albino Rat. *Grant Van Huysen, Indiana University School of Dentistry, Indianapolis, Ind.* Albino rats fed 31 days on a Hoppert, Weber, and Caniff diet were sacrificed and their heads subdivided so that each series of 3 molar teeth were held together in mandibular or maxillary tissue. No caries was found in 600 molar teeth at 12× magnification. The blocks were fixed, dehydrated, embedded in balsam, and ground on both the buccal and lingual sides to expose the fissures to about their mid-point. The sections were from 0.7 to 0.9 mm. thick. Eighty-five per cent of 285 fissures examined at 90× magnification contained plugs of soft, brown, fibrous material, assumed from its appearance to be impacted food. In spite of the corn particle diet and trauma of grinding, enamel fracture was seen in only one instance. This was not associated with caries. Three of the fissures showed caries microscopically. This caries attacked the enamel walls of the fissure close to but not quite at its apex and spread laterally toward the pulp horns. It is concluded that the teeth of the albino rat on a Hoppert, Weber, and Caniff diet do not fracture unless the cusps are first undermined by caries.

40. The Normal Discontinuity of Progression of Dental Caries. *Julian D. Boyd, Kenneth E. Wessels, and Catherine Shroeder, Colleges of Medicine and of Dentistry, State University of Iowa, Iowa City, Iowa.* Each of 212 teen-aged children received recurrent dental examinations with roentgenograms for a period of 2 years. The data were tabulated to indicate the number of newly affected tooth surfaces in each mouth for each interval between examinations, as well as the duration of periods of nondetectable advance of caries. Advance of caries occurred among most of the subjects during the 2-year period of study; the mean annual increment was 2.75 tooth surfaces. In 90 per cent of the subjects, periods of nonadvance were interspersed among periods of advancing caries. One-third of the group had consecutive periods of at least 1 year during which no detectable evidence of newly carious lesions could be demonstrated; in more than 80 per cent of the group there were consecutive periods of 6 months or longer without the appearance of new lesions. In 60 per cent of the group, an aggregate of 8 months or longer was noted with no new caries. The experimental group was divided into quartiles in terms of the relative net rate of caries advance. The quartile with the least advance had the highest mean consecutive and aggregate duration on nonadvance of caries. But among the quartile with the greatest caries advance, a median aggregate duration of 7 months without caries advance was demonstrated; the

middle one-half of values for that quartile ranged from 6 to 12 months. When the duration of nonadvance was compared with the number of tooth surfaces affected by decay at the first observation, no difference could be noted between the highest and lowest quartile. The median duration on nonadvance was the same for each: 11 months. The range of values for the contrasted quartiles also was the same for the middle one-half of each of these groups: from 6 to 17 months. It is to be emphasized that one would have been misled if he had attempted to correlate the incidence or the duration of nonadvance of tooth decay with any concurrently imposed regimen. Test studies thus demand the determination of freedom from advancing caries in a noncontrolled population of like attributes before conclusions are drawn or implied. From these findings it must be assumed either that the evolution of dental caries is discontinuous, or else that there are periods of latency prior to the time when a carious area can be detected clinically or roentgenographically.

VII. Fifth Session: Saturday Evening, March 25; Abstracts 41-49

41. A Study of the Masticatory Forces of a Denture Wearer. *Finn Brudevold, Tufts College Dental School, Boston, Mass.* The vertical forces used in chewing 19 different foods, which included meats, fish, fruits, vegetables, and cereals, were measured in a denture wearer. The total forces required to chew a mouthful of food varied from 72 kg. for dried apricot to 271 kg. for raw carrot. The greater force was produced primarily by increasing the number of chews rather than by increasing the force of a single chew. The mean force of a chew was in the range of 0.6 to 1.6 kg. on the first molar and the magnitude was somewhat less on the premolars. Tough foods were shifted forward toward the first premolar during chewing. Other types of foods were chewed to a great extent on the second premolar and on the first molar. A slight soreness on the alveolar ridge under the denture base reduced the chewing force by one-half. Opposing teeth did not make contact with any appreciable force during chewing. A comparison of the chewing forces recorded in the present study with the forces required to crush food in vitro using hinge or shearing movements suggested that the denture wearer chewed with a shearing movement.

42. The Pathogenesis of Desquamative Gingivitis: A Disturbance of the Connective Tissue Ground Substance. *Milton B. Engel, Harold G. Ray, and Balint Orban, University of Illinois, College of Dentistry, Chicago, Ill.* A series of 8 patients with desquamative gingivitis was studied. All but 2 of the group were women. Gingival biopsies were fixed by the freezing-drying method. The tissues were infiltrated with paraffin and sectioned. The paraffin was dissolved in petroleum ether and the sections were then stained by the periodic acid-leucofuchsin method to demonstrate glycoproteins and other carbohydrate containing components. In desquamative gingivitis the basement membrane was either absent or discontinuous and the connective tissue ground substance was only weakly stained. The connective tissue fibers appeared as though unmasked by the solution of the surrounding ground substance. In some areas the glycoprotein cementing substance of the epithelial cells was dissolved. Solubility tests done on the sections with 0.15 M phosphate buffer (pH 7) before and after alcohol denaturation indicated the presence of relatively large amounts of an alcohol-insoluble, water-soluble carbohydrate-containing fraction in the connective tissue ground substance. Normal gingivae contain only small amounts of this fraction. These findings suggested the interpretation that the gingival tissues in desquamative gingivitis produce abnormal quantities of enzymes which

depolymerize the connective tissue ground substance. It is proposed that this enzymic activity is mediated in some manner by the female sex hormones. (Published in *J. D. Res.* **29**: 410, 1950.)

43. Salivary Gland Tumor Induction by Chemical Carcinogens. *John J. Byrne and William H. Bauer, Saint Louis University School of Dentistry, Saint Louis, Mo.* Much of the confusion existent in classification of salivary gland tumors is a result of an incomplete concept of the genesis of these tumors. Study of experimental tumor origin could shed considerable light on the mode of the development of human salivary gland tumors. Rabbits and various strains of mice were studied to determine the effects of several carcinogenic implants. The parotid glands of rabbits and the parotids and submaxillary glands of C57 black, AK, and C mice were the sites of implantation. Twenty implants were made in rabbits using methylcholanthrene, 9-10 dimethyl-1,2-benzanthrene, 1,2-benzanthrene and 1,2,5,6-dibenzanthracene. Four animals were sacrificed for microscopic study at periods of 3, 6, 9, and 12 months. No metaplastic or neoplastic changes of ductal epithelium were observed. One parotid containing an 11-month-old methylcholanthrene implant revealed proliferation of intercalated duct epithelium. Other glands showed transformation into a more or less dense fibrous tissue around well-preserved pellets. Of the various strains of mice tested for carcinogenic sensitivity, AK and C were the more responsive to tumor development, C57 being somewhat resistant and often demonstrating intoxication. Microscopic examination of the tumors seemed to indicate that the cuboidal epithelium of the intercalated ducts reacted by proliferating, while still maintaining its cuboidal shape and arranged itself in acini, thereby forming adenomas. Between these glandular formations were areas where epithelial cells separated in a mucinous matrix which staining reactions indicated was "pseudo cartilage" as seen in "mixed tumors." Various metaplastic changes occurred in the epithelium, some without manifesting malignancy. Others demonstrated papillary cystadenomas, papillary adenocarcinomas, squamous cell carcinomas, and carcinosarcomas. Because of rapid growth the sequence of neoplastic development was difficult to follow and is being studied in another series of experiments.

44. The Structure of Enamel Matrix. *Harry Sicher, Dental School, Loyola University, Chicago, Ill.* In the last years the concept that each ameloblast is responsible for the formation of one individual enamel rod has been repeatedly denied. It has been claimed that the ameloblasts elaborate a primarily homogeneous matrix in which the prisms differentiate at a later stage; it has even been claimed that the course of the rods may change during or preceding the phase of enamel maturation. It can be shown that the alleged homogeneity of young enamel matrix is merely the consequence of equal affinity of rod and interrod substance to certain stains, for instance, hematoxylin. In sections stained with Mallory azan, the enamel rods are visible as separate elements from the very first beginning of enamel formation and appear in exactly the same position and relations that persist through formative and maturing phases.

45. Are Human Teeth Degenerating? *Peter J. Brekhus, School of Dentistry, University of Minnesota, Minneapolis, Minn.* In 1929, 1939, and 1949, respectively, the teeth of freshman students entering the University of Minnesota were examined, and the prevalence of dental caries recorded. Findings indicate that the amount of caries has been increasing progressively during the 20-year period. In 1929, a total of 3,388 students, both male and female, averaged 9.95 teeth affected by caries. Ten years later, 4,348 students showed an

average of 11.8 teeth affected. In the 1949 study, 4,412 freshmen averaged 13.7 teeth affected by caries. All the differences are statistically significant. The average number of fillings per student was 7.8 in 1929, 8.8 in 1939, and 11.4 in 1949. The average number of extractions per student was 0.70 in 1929, 0.78 in 1939, and 1.11 in 1949. Among the students examined in 1929, only 1.8 had teeth entirely unaffected by caries. In the 1939 group, this percentage was 1.15, and among the 1949 freshmen, examiners found only 0.68 per cent with no caries. No significant differences were found between the men and the women in any of the three studies. A consistent pattern was observed in the teeth most affected by caries. In each of the studies, these were the first molars, the second molars, the second premolars, and the first premolars, in that order. In contrast with the apparent progressive degeneration of the teeth is the constantly improving physique of the average student. The 1939 group of freshmen were taller and heavier than the 1929 group, and there was a similar increase in height and weight in the 1949 group as compared with those examined in 1939. No obvious reason can be assigned for the decline in dental resistance. It is to be hoped that these studies will be continued at 10-year intervals, so that in time the possible emergence of a "trend" may be established.

46. The Effect of Parathyroid Hormone on the Skeleton and Dentition of *ia* Rat. *S. N. Bhaskar, I. Schour, R. O. Greep, and J. P. Weinmann, College of Dentistry, University of Illinois, and Research Laboratories, Harvard School of Dental Medicine, Boston, Mass.* The present study is based on a roentgenologic and histologic analysis of 14 *ia* (incisor-absent) rats which were given 125 to 690 rat units of parathyroid hormone beginning at 2 to 9 days of age for a period of 6 to 37 days. Fifteen untreated *ia* littermate rats were used as controls. The *ia* strain of rats is characterized by a genetic deficiency in bone resorption leading to osteosclerosis, odontome formation, and failure in tooth eruption (Schour, Bhaskar, Weinmann, and Greep: *Am. J. Anat.* **85**: 73-111, 1949). The osteosclerosis is not limited to the alveolar bone, but affects the entire skeleton. (Bhasker, Weinmann, Schour, and Greep: *Am. J. Anat.* **86**: 1950). Parathyroid hormone was administered in an attempt to increase bone resorption. This treatment reduced the incidence and severity of the odontomes and of osteosclerosis. The effect was directly related to the amount and time of administration. Tooth development during the experimental period became normal and some of the incisors and molars finally erupted.

47. Electron Microscopy of Dentin. *David B. Scott, National Institute of Dental Research, and Ralph W. G. Wyckoff, Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.* Electron micrographs are presented which demonsrate: isolated dentinal fibers, the fine structure of their walls, and their apparent contents; collagenous fibrils in the matrix; and other ultrafine fibrous components of the dentin. Two types of preparation have been studied: shadowed collodion replicas of undecalcified dentin, and thin sections of decalcified dentin. (Published in *J. D. Res.* **29**: 556, 1950.)

48. Surface Studies of Teeth From Alaskan Eskimos and West Greenland Natives. *Poul O. Pedersen, David B. Scott, National Institute of Dental Research, and Ralph W. G. Wyckoff, Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Md.* Metal shadowed collodion replicas for optical microscopy have been prepared from the surfaces of teeth from Alaskan Eskimo skulls (Smithsonian Institution, Washington, D. C.), and from extracted teeth of present-day West Greenland natives (Julianehaab District). Microscopic surface characteristics have been studied and compared with those observed on surface replicas taken from teeth of present-day American

whites. The most striking difference noted was a marked irregularity in the distribution and course of the perikymata on the Eskimo tooth surfaces.

49. Premature Aging in Dentin. *Bruce O. Hord, Faculty of Dentistry, University of Toronto, Toronto, Ontario.* Review of the literature and study of many ground sections of human teeth of various ages gave the following information regarding the character of aged dentin. Bodecker and Applebaum have made mention of the two-way flow of dental lymph along the dental tubuli. These tubuli are reasonably unobstructed in the dentin of young teeth. As the permeability of these tubuli becomes decreased by the deposition of calcium salts on the inner walls of the tubuli, the flow of dental lymph is retarded. This results in a drying and crystallation of the fluid lymph. A calcific plug forms within the tubule. The remaining fluid lymph between the adjacent calcific plugs decomposes to carbon dioxide. This is a probable explanation for the intermittent dark and light bands in metamorphosed dentin. The dark areas are the gaseous regions and the light areas mark the regions of calcific plugs. Transparent or sclerotic dentin probably forms by a process of calcification by way of the lateral anastomosing branches of the dentinal tubuli. The anterior teeth of living dogs were selected for this investigation. Small labiogingival cavities were cut approximately 1 mm. into the dentin of these teeth. One-half of the cavities were sealed with a 3 per cent paraformaldehyde paste while the remaining teeth were sealed with a zinc oxide eugenol paste to serve as a control series. At the end of 8 weeks the animals were sacrificed and the teeth removed from the jaws. Serial microscopic ground and decalcified sections were made from these specimens. Careful examination of these specimens revealed the following: (1) Three per cent paraformaldehyde produced a localized aging of the primary dentinal tubuli underlying the base of the cavity preparation. (2) Since aged dentin is less sensitive than young permeable dentin, we feel sure that these localized tracts of aged dentin are less sensitive than the unaltered dentin beyond the confines of the cavity preparation. (3) The drug was found to be irritating within physiologic limits when used in shallow cavity preparations and not exceeding a concentration of 3 per cent. No evidence of hyperemia, fibrosis, or devitalization of the delicate dental pulp was observed. (4) Control samples revealed no aging or secondary dentin formation. There was no significant change within the dental pulp.

VIII. Sixth Session: Sunday Morning, March 26; Abstracts 50-60

50. The Use of Microwave Diathermy in Dentistry for the Treatment of Swelling and Trismus. *R. Quentin Royer, K. G. Wakim, Stanley A. Lovestedt, and Frank H. Krusen, Mayo Foundation, Graduate School, University of Minnesota, Rochester, Minn.* Microwaves are a form of electromagnetic energy and are designated by a certain range of waves in the radio-frequency spectrum. Microwaves can be generated by a multicavity magnetron tube which produces radio-frequency energy at 2,400 megacycles per second at wave lengths of approximately 12 cm. The use of microwaves has been shown to be a very convenient method for heating of tissues by which we can obtain an accurate localization by direction of a beam of energy on any surface of the body. Apparently microwaves can be safely used to heat any tissue of the human body except the eye, where deleterious effects have been observed. An externally applied, penetrating, and focusable heat may help to alleviate the swelling and trismus which follow third molar odontectomy by causing a rise in the temperature of the tissues, and increasing the rate of the local circulation, fluid exchange, and lymph drainage. The swelling and trismus which followed bilateral third molar odontectomy was measured in 35 patients. It will be shown

that microwaves applied to the external surface of the jaw can effect an average rise in the intraoral temperature of 0.9° C. on the lingual side of the mandible and an average rise of 1.1° C. on the mucosa lateral to the site of extraction.

51. Aureomycin: Its Use in the Treatment of Acute Dental Infections in Children. A Preliminary Report. *Gordon H. Rovelstad, Northwestern University Dental School, Chicago, Ill.* Aureomycin has proven itself to be one of the most versatile of the newer antibiotics. Because of its effectiveness, low toxicity, and ease of administration, its use in the treatment of acute infections of dental origin should have great potentialities. Patients presenting with acute dental infections to the dental clinic of Children's Memorial Hospital of Chicago have been treated with aureomycin by oral administration for the past 6 months. To date, all cases thus treated (32) have shown a subjective and objective recovery within 24 to 48 hours and a complete recovery within 3 to 5 days.

52. Use of 9-Aminoacridine Hydrochloride Solution in Endodontia. *Lester E. Breese, University of California, College of Dentistry, San Francisco, Calif.* The drug was used for pulp canal and periapical sterilization. Roentgenograms of apical areas before, during, and after treatment were demonstrated. Technics and indications for use were discussed as were possible allergic reactions.

53. Window Technic for Enamel Solubility Studies. *Thomas M. Boyd, Indiana University School of Dentistry Indianapolis, Ind.* This study describes how the enamel surface of the tooth may be used instead of powdered enamel for the study of the solubility of its calcium and phosphorus. Extracted maxillary first incisor teeth stored temporarily in tap water are superficially cleaned with a mixture of $CaCO_3$ and fine pumice. The roots are removed, and the pulp chamber is enlarged. The crown is cemented onto the end of a 6-inch length of 14-gauge wire. A 5.5 mm. lead foil disc is loosely adapted to the central portion of the labial surface of the crown preparation. A thin, creamy mix of powdered gilsonite and toluene is carefully applied over the entire surface of the crown preparation. The lead foil disc is then carefully dissected away, exposing a window of enamel surface. This is suspended from a rubber stopper in a test tube so that the exposed enamel is decalcified by immersion in 20 ml. of 0.2 N acetic acid, pH 2.5. The sealed tube and contents are rotated in a water bath at 37° C. for 24 hours. The calcium and phosphorus content of the acetic acid is determined by the methods of Clark and Collip and Fiske and Subbarow, respectively.

54. A Volumetric Method for Measuring Masticatory Performance With Different Test Foods. *Algirdas Yurkstas and Richard S. Manly, Tufts College Dental School, Boston, Mass.* The purpose of the investigation was to develop a masticatory performance test which would be generally applicable to a wide variety of foods, to apply this to a representative number of foods, and to select one or more which would be a good index of ability to masticate tough foods. The gravimetric method previously reported (Manly and Braley) was not suitable for the majority of foods. The procedure was changed to permit volumetric measurements, and this was found to be applicable to 35 out of 40 foods tested. The masticated food was sieved on 5- or 10-mesh screens and the volumes retained and passing were measured in calibrated centrifuge tubes after centrifuging. The standard deviation of repeated tests was approximately 2 per cent, and the results with peanuts as a test food agreed with the gravimetric procedure. Tests with 35 different foods were performed by the same individual at 20 chews, and the chewed food was subjected to sieve analysis on 4-, 10-, 20-, 40-, and 200-mesh screens. The particle size distribution was plotted on logarithmic-probability paper. The distribution was a curve for 34 foods,

indicating that mastication is selective in causing more rapid grinding of the coarser particles. This confirms a similar finding for peanuts (Manly and Braley) and establishes it as a generalization. The resistance to pulverization for peanuts was about the same as the average for 11 varieties of nuts. Raw carrots and dried prunes were found to be the most representative tough foods among 11 fruits and vegetables. Boiled ham seemed to be the most satisfactory representative of 11 varieties of fish and meats.

These 4 foods were evaluated on 10 half-dentitions at 10, 20, 40, and 80 chews. The logarithm of the number of chews was found to be proportional to the probability function of the performance. The relative resistance to pulverization was found to be carrots > ham > prunes > peanuts, and the order was maintained throughout the chewing period. Carrots required 8 times as many strokes as peanuts for pulverization to the same particle size, and ham required 4 times as many chews.

55. Electronic Device for Reducing Mechanical Vibrations Accompanying Dental Preparations. *C. A. Scrivener and W. D. Bemmels, Ottawa University, Ottawa, Kansas.* This electronic device is designed to eliminate or reduce mechanical vibration incidental to the operation of dental burs and stones. A pickup microphone of proper design is attached to the source of the vibration, that is, to the dental handpiece or to the tooth or instrument which produces the vibration or to the tissues adjacent thereto. The electrical impulses produced in the microphone are carried into an amplifier, which is capable of increasing them with a negligible amount of harmonic, phase, and frequency distortion. They are then carried through a device known as a phase inverter which inverts the vibrations 180°. The phase may be inverted by several different means, the simplest of which is merely to reverse the lead wires from the amplifier. From the phase inverter the electrical impulses are carried to a transducer of the proper type, where they are converted into vibrations and are delivered back to the source of the original vibration or to the adjacent structures or organs in intensity and frequencies identical with, but at 180° phase relation to, the original vibration. The two vibrations (the original and the induced countervibration), moving in perfect synchronization in opposite directions, tend to neutralize or nullify the influence of each other.

56. An Instrument for Measuring Tooth Mobility. *Richard S. Manly, Algirdas Yurkstas, and James B. Reswick, Tufts College Dental School, Boston, and Massachusetts Institute of Technology, Cambridge, Mass.* During the past three years, an instrument has been developed for measuring tooth mobility precisely and objectively. The principle involved the measurement of the resistance of teeth to vibration (elastic constant) at a certain frequency. The equipment consisted of an audio-frequency signal generator, phonograph record cutter and pickup, amplifier and A.C. voltage meter. A rod was driven by the cutter at frequencies ranging from 100 to 10,000 cycles per second, and the amplitude measured by the pickup, amplifier, and meter. The resistance of a tooth to motion was read from the decrease in amplitude of the vibrating rod when it was touched to the tooth. An attempt was made to find the resonant frequencies for teeth having different clinical mobilities. No sharp peaks were observed. Next, a series of similar extracted teeth were coated on the root portion with different thicknesses of gutta-percha, rubber, and Dicor, then set in dental stone with just their anatomical crowns exposed. The instrument was set into vibration at frequencies from 100 to 10,000 cycles per second, and touched against the teeth. The elastic constants of the supporting structures of different teeth were observed at various frequencies. The best differentiation between teeth with various degrees of mobility was shown at approximately 1,000 cycles.

Periodontal pockets were simulated by cutting away stone from the necks of these teeth. Readings at 1,000 cycles were found to offer the best indication of pocket depth.

The initial apparatus was too much influenced by hard pressure for clinical use. After several trials, this difficulty was overcome sufficiently to permit development of a clinical model, which was applied to 233 lower incisors, canines, premolars, and first molars. Thirty-seven of these had clinical mobilities of 1 to 3 mm. The instrument readings were well correlated with clinical mobility.

57. Dentinal Tubules as a Route for the Transmission of Poliomyelitis Virus and Horse Serum. *William David McBride and Jerome T. Syverton, University of Minnesota School of Medicine, Minneapolis, Minn.* Dentinal tubules have been reported to transmit horse serum to the pulpal tissues and have been suggested as a route for the passage of poliomyelitis virus. Passage of virus particles through dentinal tubules was tested by employing a 10 per cent suspension of poliomyelitis infected spinal cord for the inoculation of 24 cavities prepared in the labial surface of the incisor teeth of 4 monkeys and by employing a suspension of V virus for the inoculation of 2 cavities in 1 monkey. Six cavities prepared in the labial surfaces of the incisors of 3 virgin female guinea pigs were inoculated with horse serum, and 6 similar cavities in 3 additional guinea pigs were inoculated with egg albumin. Four additional cavities prepared in the incisal edges of the incisors of 2 guinea pigs were inoculated with horse serum. Passage of the viruses as measured by infection and of the protein substances as measured by proven hypersensitivity of the uterine muscles, as shown by the Schultz-Dale phenomenon, failed to occur in all cases but 1: hypersensitivity was proven for a single guinea pig that received the inoculum in the incisal edges of its teeth. It does not seem probable that dentinal tubules can serve as a route for passage of macromolecular substances, even though tracts of organic pulpal remnants, as in the incisors of rodents, may do so.

58. Pulp Reactions to Varying Temperatures. *Vincent F. Lisanti and Helmut A. Zander, Tufts College Dental School, Boston, Mass.* This is a study of pulp reactions to various temperatures for different lengths of time. Cavities were prepared in dogs' teeth minimizing frictional heat. Electrically controlled temperatures were then introduced on the dentin surface of the cavity for specified time intervals. Temperatures used were from 98.6° F. to 600° F. The animals were sacrificed 2½ to 4 hours; 24 hours; 1 week; and 2 months later. Histological sections were prepared from the teeth. Temperatures up to 150° F. did not create significant lesions; 300° F. at increasing time exposures produced increasingly greater damage including a zone of demarcation. Healing and secondary dentin became visible after one week. At 600° F. for all exposure intervals, a zone of demarcation was produced with edema, blistering, sterile and hemorrhagic necrosis.

59. The Reaction of Rat Skeletal Muscle to the Constituents of Procaine-Epinephrine Solutions in Concentrations Used for Dental Preparations. *Emmett R. Costich, University of Rochester, School of Medicine and Dentistry and the Eastman Dental Dispensary, Rochester, N. Y.* It has been observed that rats injected intramuscularly with 2 per cent procaine plus 1:50,000 epinephrine developed necrosis of the muscle at the injection site. In an attempt to determine the cause for this reaction, the constituents used in a commercial solution were prepared separately and injected intramuscularly in the rat. Four groups of 10 rats received injections of 0.25 c.c. in the thigh muscle of both hind legs. Group I received procaine 0.02 Gm./c.c. (pH 6.3); Group II, epinephrine 0.00002 Gm./c.c. (pH 4.8); Group III, sodium bisulfite

0.0015 Gm./c.c. (pH 4.3); and Group IV, normal saline (pH 6.1). One-half of the animals in each group were sacrificed at 48 hours, the other half at 96 hours. Histological preparations were made of the injected areas. Normal saline showed no reaction at 48 or 96 hours. Procaine produced an acute localized inflammation with necrosis at 48 and 96 hours. Epinephrine was similar to procaine in its tissue reaction but more diffuse and less acute. Sodium bisulfite showed only a mild reaction in 2 animals, the remainder being negative. This seemed to indicate that pH was not a factor in the tissue damage, that the reaction from epinephrine may have been due to local ischemia and that 2 per cent procaine is toxic to rat muscle.

60. Study of the Cornification of the Oral Epithelium by the Use of Oral Scrapings: A Preliminary Report. *S. C. Miller, A. Soberman, and S. S. Stahl, College of Dentistry, New York University, New York, N. Y.* A number of studies regarding the structure of the oral epithelium have been carried out. In most instances, biopsies were taken for histologic analysis of the epithelium. In this study, scrapings of exfoliating epithelial cells of selected areas of the oral mucosa were utilized. These cells were stained with the differential papanicolaou cytological stain. Male students, aged 20 to 30 yrs., were used for this investigation and four areas were selected for study. The scrapings were taken with a wooden cervical spatula (Ayre's spatula) from clinically normal appearing sites. The slides were analyzed according to type of cell found and a random cell count of 100 cells for each area was carried out. Results were: Dorsal surface of tongue (60 students); average distribution, 86 per cent acidophilic cells and 14 per cent basophilic cells. Ventral surface of tongue (50 students): average distribution, 21 per cent acidophilic and 79 per cent basophilic cells. Cheek area (50 students): 73 per cent acidophilic and 27 per cent basophilic cell distribution. Anterior gingivae (50 students): 87 per cent yellow-appearing cells and 13 per cent acidophilic cells. All cells studied appeared to come from the superficial and intermediate layers of the epithelium. Basal cells were never observed. The apparent constancy of cellular distribution in each of the areas studied seems to suggest a fairly regular cornification pattern of the oral mucosa in healthy young males. This pattern may be useful in providing a normal base line for the study of aberrations in cornification. The degree of cornification in the areas studied was highest at the gingiva, followed by the dorsal surface of the tongue, the cheek area, and the ventral surface of the tongue as has been pointed out by previous investigations. (To be published in *J. D. Res.*)

IX. Seventh Session: Sunday Afternoon, March 26; Abstracts 61-69

61. A Histologic Study of the Reaction of Skin and Mucous Membrane to a Single Topical Application of the Carcinogen 9,10 Dimethyl-1,2, Benzanthracene. *Barnet M. Levy, Robert Gorlin, and Robert Gottsegen, Columbia University, School of Dental and Oral Surgery, New York, N. Y.* Under Nembutal anesthesia, the mucous membrane and skin surfaces of the lower lip of 45 Swiss albino mice, age 2 months, were painted with a 0.6 per cent solution of 9,10 dimethyl-1,2 benzanthracene in benzene using a No. 9 camel's hair brush. Animals were sacrificed at intervals of from 1 to 35 days following the application of the carcinogen. In order to bring out the potential mitotic activity of the cells, the animals received 0.025 mg. of colchicine 4 hours before death. Hematoxalin and eosin, periodic acid, a trichrome, Wilder's silver and alkaline phosphatase studies were made on all sections. The changes in the skin were consistent with those reported by Cramer and Stowell (*J. National*

Cancer Institute, 1942). Changes in the mucous membrane are increased amounts of surface keratin and increase in size and number of granules in the stratum granulosum. Some of these granules extend into the stratum spinosum. There is a slight hyperplasia of the epithelial cells, the proliferation being most marked in the stratum germinatavum. There are hydropic changes in the cells of the stratum spinosum and the stratum granulosum. The lamina propria becomes thickened by edema and a fine, fibrinous precipitate. There is no difference in alkaline phosphatase activity in skin or mucous membrane between the painted, untreated, and benzene-control animals.

62. Fluorine Content of Teeth. *F. J. McClure and R. C. Likins, National Institute of Dental Research, National Institutes of Health, Bethesda, Md.* The fluorine content of human dentin and enamel is being studied in relation to the fluorine in the drinking water. Where the fluorine in drinking water does not exceed 0.2 to 0.3 ppm, enamel and dentin may be expected to contain approximately 0.0100 per cent and 0.0230 per cent fluorine, respectively. A continuous exposure to 1.0 to 1.1 ppm fluorine in drinking water resulted in approximately 0.0135 per cent fluorine in the enamel and 0.0385 per cent fluorine in dentin. An average of 0.0650 per cent fluorine in enamel and 0.1250 per cent fluorine in dentin was associated with a maximum of 7.6 ppm fluorine in drinking water. Quantities of fluorine in drinking water between these two extremes added proportional amounts of fluorine to human dentin and enamel.

63. Observations of Salivary Lactobacillus Counts for a Period Before and After Topical Applications of Two Per Cent Sodium Fluoride. *Faul C. Kitchin, Hamilton B. G. Robinson, Dorothy Permar, College of Dentistry, Ohio State University, and Andrew H. Imhoff, Dental Division, City Department of Health, Columbus, Ohio.* A total of 77 children, aged 7 to 9 and 14 to 16 years, were divided into experimental and control groups. The experimental groups received 4 topical applications of 2 per cent sodium fluoride, the first preceded by an oral prophylaxis. The control received only an oral prophylaxis. Salivary lactobacillus counts were taken before and at intervals after the treatments for 1 year for the 14- to 16-year age group, and for 2 years for the 7- to 9-year age group. The counts showed no consistent differences; no changes were observed in the salivary lactobacillus counts of the experimental group that were not of an order equal to those of the control groups.

64. Preliminary Studies of the Caries Inhibitory Potential and Acute Toxicity of Sodium Monofluorophosphate. *John W. Hein, Kanwar L. Shourie, and Harold C. Hodge, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Little is known concerning the forms in which fluorine may be administered to be effective against dental decay. All studies to date have dealt with compounds which dissociate to some extent in water to yield the fluoride ion. It was of interest, therefore, to test the ability to inhibit caries activity and the acute toxicity of a complex fluoride, monofluorophosphate, which does not yield fluoride ions in aqueous solution. Three littermate groups of 10 male and 10 female hamsters, on a caries-producing diet, were given 3 different drinking solutions: Group I, distilled water; Group II, 40 ppm F. as NaF; Group III, 40 ppm F. as Na_2PO_3F. The resulting total caries scores after 113 days were: Group I, 63,22; Group II, 2,4; Group III, 1,4 for males and females, respectively. The acute oral toxicity of NaF and Na_2PO_3F was determined for albino rats weighing 200 to 300 grams. The acute toxicity of Na_2PO_3F was also determined for intraperitoneal injection on similar animals. Comparison of predicted LD50's showed Na_2PO_3F to be 7 to 8 times less toxic than NaF. On the basis of fluoride content the complex fluoride is 2.3 to 3 times

less toxic than the free ionic form. Albino rats fed 0.2 per cent Na_2PO_3F. (equivalent to 0.058 per cent NaF) for 30 days did not show changes in incisors equal to those reported for 0.025 per cent NaF.

65. The Evanston Dental Caries Study. VI. A Comparison of Prefluoride and Postfluoride Caries Experience of 6-, 7-, and 8-Year-Old Children in the Study Area (Evanston, Ill.). *I. N. Hill, J. R. Blayney, O. E. Jelinek, and W. Wolf, Walter G. Zoller Memorial Dental Clinic, Chicago, Ill.* The available data has been analyzed in an effort to determine if a significant change in the caries prevalence rates in the 6-, 7-, and 8-year-old age group has occurred following the exposure to Lake Michigan water containing 1.0 ppm fluorine for a period varying from 12 to 22 months. Findings to date appear to indicate that the 6- and 7-year-old children have a lower incidence of caries immunity while the 8-year-old age group experiences a higher incidence of immunity when compared with the base line or prefluoridation period. The caries experience rate per 100 teeth of the occlusal surfaces of the first permanent molar is less in the postfluoridation group. It seems apparent that a longer period of study is required before highly significant findings can be reported. (Published in *J. D. Res.* **29**: 534, 1950.)

66. The in Vivo Effect of Fluoride on Carbohydrate Metabolism. I. The Effect on Blood Sugar Levels. *G. W. Rapp, Loyola University School of Dentistry, Chicago, Ill.* Administration of fluoride in doses resulting in a blood concentration of from 2 to 10 ppm. results in hyperglycemia. This hyperglycemia resembles that produced by epinephrine. The rate of absorption of glucose from the small intestine is considerably delayed. All of these effects tend to illustrate that the effect of fluoride on carbohydrate metabolism is as a poison for the phospho-esterase enzymes.

67. An Attempt to Produce Sulfamylon-Resistant Strains of Microorganisms *B. F. Gurney and Barbara Griffiths, Loyola University School of Dentistry, Chicago, Ill.* Sulfamylon as an antiseptic and germicide, particularly in root canal therapy, has proved its worth. There is the ever-present danger of producing drug fastness in microorganisms when amounts of drug as small as can be put into root canals are used. Because of the fact that Sulfamylon does not have the same mode of action as the regular sulfa drugs, this study is of both academic and practical value.

68. Some Observations on the Action of Saliva on Sucrose. *J. F. Volker, School of Dentistry, University of Alabama, Birmingham, Ala.* It has been shown that when sucrose is added to the diet of the Syrian hamster, it is at least as effective as glucose in causing experimental caries. Furthermore, it has been demonstrated that immediately following the ingestion of sucrose in vivo enamel surface acid production occurs. These considerations point to the importance of understanding the mechanism of oral sucrose degradation. The conversion of sucrose by salivary enzymes to reducing substances, presumably glucose, has been the subject of in vivo and in vitro study. Varying quantities of sucrose have been added to pooled salivary samples and, at intervals from 1 minute up to 2 hours, aliquots have been analyzed for the presence of glucose. With this method it has been possible to show the immediate appearance of glucose in saliva-sucrose mixtures and its persistence at approximately the same level during the duration of the experiment; for example, if 1 per cent sucrose is added to 10 c.c. of fresh saliva, the reducing substance concentration increases from 15 mg. per cent to 70 mg. per cent within the first 2 minutes and remains at this level for the duration of the experiment (30 minutes). If 10

per cent sucrose is added to 10 c.c. of pooled saliva, an increase in reducing substance concentration from approximately 15 mg. per cent to 200 mg. per cent occurs within 2 minutes and persists for the duration of the experiment (30 minutes). Similarly, if 1 Gm. of a ration containing 25 per cent sucrose but no glucose is ingested, a salivary glucose level of 150 mg. per cent is found within 2 minutes.

69. The Effect of Fatty Acids on the In Vitro Growth of Some Oral Bacteria. *Robert M. Stephan, National Institute of Dental Research, National Institutes of Health, Bethesda, Md.* Oleic acid in low concentrations has been shown to be a growth factor for certain lactic acid bacteria (Williams, W. L., Broquist, H. P., and Snell, E. E., *J. Biol. Chem.* **170**: 619, 1947) and several fatty acids in higher concentrations are known to inhibit the growth of bacteria. Using an auxanographic culture plate method, a study has been made of the effects of a series of fatty acids on (1) the growth of some oral microorganisms, and (2) on their ability to dissolve calcium phosphate precipitated in a semidefined agar culture medium. The fatty acids included oleic, linoleic, undecylenic, and a homologous series of straight chain saturated acids from C_2 to C_{18}. The microorganisms included pure cultures of 25 strains of lactobacilli, strains of staphylococcus and streptococcus, and the mixed oral flora from plaques and saliva. Under the conditions of these experiments which involve diffusion through agar, the longer chain fatty acids (C_{14} to C_{18}) produced only small zones of inhibition of growth, or none at all. However, the alkaline salts of the C_{14} to C_{18} acids produced more definite inhibitory effects. Extensive zones of inhibition of growth were produced by the C_6 to C_{12} acids. All of the microorganisms showed some susceptibility to the inhibitory effects of the C_6 to C_{12} acids. Slight stimulatory effects were observed for some of the lactobacilli with oleic and linoleic acids. A direct dissolution of calcium phosphate precipitated in the culture medium was observed with the C_2 to C_9 fatty acids. With longer chain length fatty acids, no direct dissolution effect was visible; sometimes an apparent retarding of the dissolution of the precipitate by bacterial action was observed.

X. Materials Group: Saturday and Sunday, March 25 and 26; Abstracts 70-82

70. Hardness and Flow of Gypsum Materials. *D. B. Mahler, University of Michigan, School of Dentistry, Ann Arbor, Mich.* This report discusses the analysis of gypsum materials as related to the resistance to penetration of a small indenter. Rockwell hardness values for these materials as well as a test for determining their flow properties utilizing the Rockwell Superficial Hardness Tester was given. These properties were then related to a problem arising from the processing of acrylic resin as used in the field of complete denture prosthesis. Storage conditioning of these materials which will insure optimum properties in their application to dental usage was mentioned.

71. Some Factors Which Affect Polymerization of Acrylic Resins. *W. T. Sweeney, T. E. Fischer, and I. C. Schoonover, National Bureau of Standards, Washington, D. C.* This report was based on information obtained from the research project on the causes of certain unexplained differences in the polymerization of the same batches of methyl methacrylate resins. Data was presented to show that small amounts of chemicals such as water can have an important effect on storage and polymerization rates. The theory of acrylic resin polymerization was discussed and the question of why the reaction rates

are different when inhibitors, catalysts, and other agents are present was given some consideration. Data have been obtained which indicate that any of our practical dental difficulties can be attributed to these small amounts of chemicals. Some suggestions for eliminating these troubles were presented.

72. Clinical Observations on Contracting Amalgam Alloy. *R. E. McDonald and R. W. Phillips, Indiana University, School of Dentistry, Indianapolis, Ind.* The purpose of this study was to determine if a slight contraction in amalgam restorations could be observed clinically. The contraction was exaggerated by use of a special high tin content alloy (32 per cent) which contracted 30 microns with the manipulative technic employed. Sixty restorations were placed in the permanent teeth of 32 child patients who were between the ages of 8 to 15. Of this number, all but 6 of the restorations were examined periodically with 34 being in service for at least 3 years. There was no clinical evidence of contraction indicated, by open or carious margins, on any restoration. The results of this study seem to indicate that there is no deleterious clinical effect from the lesser degree of contraction that might be produced from slight overtrituration of an accepted amalgam alloy. (Published in *J. D. Res.* **29**: 482, 1950.)

73. Abrasions of Tooth Surface by Nylon and Natural Bristle Toothbrushes. *E. W. Skinner, Northwestern University Dental School, Chicago, Ill.* Human teeth were mounted in acrylic resin by a special technic and plane surfaced either in the enamel or dentin. The surface roughness of the sample was measured by means of the brush surface analyzer. The surface was then brushed with the appropriate toothbrush, using distilled water as the brushing medium. None of the enamel surfaces were changed, regardless of the type of bristle employed, but the nylon bristles roughened the dentin surface.

74. A Volumetric Expansion Apparatus for Waxes and Compounds. *A. L. Ware and A. R. Docking, Bureau of Standards, Australia.* A volumetric expansion apparatus particularly suitable for studying the thermal expansion of waxes and dental compounds was described. A continuous record can be obtained of the behavior of these materials over a wide range of temperatures which may include the change of state from solid to liquid. The apparatus is constructed in glass and the determination of expansion is based on the displacement of mercury along a calibrated tube. Typical curves for inlay waxes, baseplate waxes, and impression compounds were shown. The equipment will be useful in studying mixtures to assist in the formulation of dental waxes and compounds with minimum thermal contraction.

75. Dimensional Change of Agar-Agar Gel on Immersion in Various Sulfate Solutions. *E. A. Luster, University of Louisville and Whip-Mix Corporation, Louisville, Ky.* In recent years, the use of agar-agar as a dental impression material has gained increasing favor in the profession. It is far from a perfect material for this purpose, but superior to materials used previously. Inasmuch as there is a definite volume change in agar gels with time and conditions (shrinkage if exposed to air, swelling if immersed in water), it has been the custom to immerse impressions in a 2 per cent solution of potassium sulfate. It has been claimed that this solution will stabilize the agar gel against dimensional changes. A test method which yields empirical figures for comparative purposes has been set up and described (along with equipment). Using a 5 per cent agar gel, the exact dimensional change throughout a time range of from immediate pouring to 12 hours and, in some cases, 24 hours of immersion in water was measured, and the results presented in graph form.

In the same manner, the amount of shrinkage when exposed to air was measured. Then various percentage solutions of potassium sulfate (including 2 per cent) were run. Finally, many other metal sulfate solutions were compared with potassium sulfate. This work was done with a pure agar gel, inasmuch as agar is the basis of hydrocolloid impression materials. The various commercial brands of impression materials on the market undoubtedly are formulated differently and, therefore, may react differently in such a test. Inasmuch as there was not sufficient time to run all possible brands in this procedure, it was thought best to choose the raw material, keeping it constant throughout the tests. The results are most interesting in themselves as a possible means for stabilizing dental impression materials, and will be even more interesting when tried on fully formulated compounds for dental use.

76. Some Physical Properties of Direct Resinous Filling Materials. *G. C. Paffenbarger, H. C. Caul, D. L. Smith, and W. B. Wolcott, National Bureau of Standards, Washington, D. C.* Some physical properties of resinous filling materials which are pertinent to their dental application are being investigated by the American Dental Association Research Fellowship at the National Bureau of Standards. Among these properties are consistency of mix, time of hardening, temperature rise on hardening, solubility, dimensional change during polymerization, pressures necessary to prevent porosity, and clinical behavior of these resins as compared to silicate cements. On the basis of these and additional investigations to be made, an American Dental Association specification for these materials can be formulated.

77. Effect of Composition on the Physical Properties of Dental Silicate Cements. *R. S. Manly, C. F. Baker, P. N. Miller, and F. E. Welch, Tufts College Dental School and Department of Chemistry, Boston, Mass.* The properties of dental silicate cements have been studied by mixing a standard commercial powder with liquids of varying composition in a search for a cement with improved physical and chemical properties. Different concentrations of aluminum and zinc were investigated as modifiers in the liquid. Calcium, iridium, barium, beryllium, and magnesium were tried as substitutes for aluminum and zinc. Other elements were eliminated as possibilities because of insolubility, rarity, or colored sulfides. Consistency, setting time, and water solubilitiy were performed on cements prepared from the test liquid and a standard powder. Promising compositions were further subjected to hardness determinations. None of these compositions were found to be superior to cements made with a commercial liquid. Nineteen powder compositions containing CaO, Al_2O_3m and SiO_2 in various amounts, using as fluxes Na_2CO_3, NaCl, NaF, CaF_2, or B_2O_3, were prepared in a Globar electric furnace capable of attaining temperatures up to 2,800° F. Many of the powders with sufficient flux set up successfully with commercial cement liquid. All compositions which set were inferior to commercial cements in hardness and water solubility, probably because insufficient powder could be incorporated in the liquid. In order to test whether the pulpal irritation by silicates was attributable to flux in the powder, 23 fusions of CaO, Al_2O_3, and SiO_2 were made. All were glasses, and after pulverization, 13 set to a firm mass with commercial silicate liquid. Three samples with Ca-SiO_2 ratios of 0.51 to 0.56 and Al_2O_3 content of 20 to 26 per cent gave setting time of 3 to 8 minutes. A few samples tested for hardness did not compare favorably with commercial powder.

78. Some Vibrations Produced During the Operation of the Dental Handpiece. *E. E. Henry and F. A. Peyton, University of Michigan, Ann Arbor, Mich.* This report described the method used for determining vibrations produced during the operation of the dental handpiece. Some characteristic vibra-

tions in different portions of the handpiece were reported. A comparison of vibrations resulting from the use of different cutting instruments were described. The influence of such factors as operating speed and pressure, as they influence the resulting vibrations were discussed. (Published in *J. D. Res.* **29**: 601, 1950.)

79. Panographic Radiography. *R. J. Nelson, University of Washington, School of Dentistry, Seattle, Wash.* An apparatus and method has been developed which allows all the teeth of either jaw to be registered on a single film. The mechanical features of the x-ray apparatus, the film size and shape, placement of the film, and angulation and exposure factors were discussed. Representative films and slides of the apparatus were shown. The possible use of this device in public health and military dentistry was suggested.

80. Some Physical Properties of Acrylic Resins Used as Denture Base Materials. *A. C. Swaney, National Bureau of Standards, Washington, D. C.* The transverse strength and deflection of 39 brands of acrylic resin used for denture bases were determined on water-saturated specimens stressed in air and under water. The resins, on the average, were about 13 per cent weaker when stressed under water. The deflections at 4,000 gram and 6,000 gram loads were 12 per cent and 9.5 per cent less, respectively, when specimens were submerged in water during application of the stress. The temperature range to which the tissue slide of a full upper denture is subjected in service is approximately 25°C. to 41°C. when eating ice cream and drinking hot coffee. Additional experiments are in progress on solubility and water sorption. Data from these tests will be used in revising American Dental Association Specification No. 12 for Denture Base Materials. Suggestions for revision of the specification will be welcomed.

81. Effect of Certain Abrasive Materials on Tooth Enamel: Progress Report. *L. Rush Bailey and R. W. Phillips, Indiana University, School of Dentistry, Indianapolis, Ind.* The effect of various abrasive and polishing prophylactic agents upon the enamel surface of extracted teeth are reported. The methods employed for measuring surface loss and luster changes are described with preliminary data concerning several materials. Abrasiveness is correlated with application time and technic. Microscopical studies are also included.

82. Self-Polymerizing Resins for Tooth Restoration Progress Report. *J. H. Emmerson, Northwestern University Dental School, Chicago, Ill.* The specific gravity of self-polymerizing acrylic resins when cured in different volumes is being studied, together with their Knoop hardness and abrasion resistance. Clinical cases are also under observation. A preliminary report of these experiments was presented.

XI. Abstracts of Papers Read Only by Title; Abstracts 83-123

83. In Vivo Study of the Uptake of Radioactive Iodine by Human Teeth. III. *Herbert J. Bartelstone, Dental Department, Montefiore Hospital, New York, N. Y.* A diffuse in vivo penetration of human enamel, dentin, and cementum by iodine^{131} is again demonstrated. Increased uptake is shown in the enamel in the areas of caries or increased organic material in teeth in which both saliva and pulp may play a role. Evidence is also presented to show pulp to surface penetration of I^{131} through dentin and enamel with concentration at the surface of the enamel and in an area of enamel caries. Carious dentin also shows a higher uptake of I^{131} and hard stain arrested caries under a sealed amalgam filling shows increased uptake, indicating pulp to surface penetration through this carious dentin. Dentin which was impregnated with silver nitrate was permeable to I^{131} in vivo.

84. I. The Ontogenetic Course of the Fully Formed Deciduous Dentition. *Louis J. Baume, College of Dentistry, George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.* A study of the developmental changes of the deciduous dentition was made of 30 children from 3 to 5½ years of age by means of plaster reproductions. Serial measurements and examination showed that after the deciduous arches were fully formed their sagittal and transversal dimensions (measured from points not affected by attrition) were not altered except when subjected to inadequate environmental influences. Two morphological arch forms were observed; the deciduous dental arches were either continuously spaced or continuously closed. Closed arches were, on an average, narrower transversally than spaced ones. The interproximal spaces were not developmental but congenital. The spaced arches frequently showed two distinct diastemata; one between the mandibular deciduous canine and first deciduous molar and the other between the maxillary deciduous second incisor and deciduous canine; they were called "primate spaces." The terminal plane of the arches in occlusion remained constantly straight in 80 per cent and in 15 per cent formed a mesial step. Vertical growth of the alveolar processes due to the development of the successional tooth germs was demonstrated by means of superimposed transverse diagraphic tracings of the same dentition at different ages. The appearance of postmolar areas upon the development of accessional teeth disclosed sagittal growth. None of the observations made in this study could corroborate the concept of physiologic changes of the deciduous dental arches through spacing and mesial shifting of the mandibular teeth.

85. II. Ontogenetic Study of the Mechanism of the Occlusal Adjustment of the First Permanent Molars. *Louis J. Baume, College of Dentistry, George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.* The development of occlusion was studied in 60 children. Through comparative measurements of serial plaster reproductions of their dental arches before, during, and after the eruption of the first permanent molars, 3 different mechanisms of normal occlusal adustment were found: (1) the occurrence of a terminal plane forming a mesial step in the deciduous denture allowed the first permanent molars to erupt directly into proper occlusion without altering the position of the neighboring teeth; (2) the presence of a mandibular primate space and a straight terminal plane was conducive to proper molar occlusion by means of an early mesial shift of the mandibular deciduous molars into the primate space upon eruption of the lower first permanent molar; and (3) closed deciduous arches and a straight terminal plane resulted in a transitory end-to-end relationship of the first permanent molars. Proper occlusion was effected through a late mesial shift of the mandibular permanent first molars subsequent to the shedding of the deciduous second molars. Migration of the lower deciduous and permanent molars constituted the physiological measure to secure proper occlusion of the permanent molars. There was no mesial shift of the mandible as indicated by the unchanged anteroposterior relationship of the opposing deciduous canines. Function and abrasion had less bearing on the molar adustment than widely assumed. The anatomic pattern of the deciduous arches controlled the ability of the deciduous and permanent molars to migrate mesially as a result of the eruptive force of the accessional teeth.

86. III. Ontogenetic Study of the Physiologic Alignment of the Permanent Incisors. *Louis J. Baume, College of Dentistry, George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.* The comparative and biometrical examination of serial casts made

of 60 cases before, during, and after eruption of the permanent incisors demonstrated the following findings: Expansion of the dental arches in the anterior region, to accommodate the larger successional incisors into proper alignment, was brought about by a lateral and frontal alveolar growth during the time of the eruption of these teeth. The mean increase in intercanine width was greater in the upper arches than in the lower ones, and again greater in previously closed upper or lower deciduous arches than in previously spaced ones. In the mandibular arches the strongest impulse of lateral growth was noted during the eruption of the second incisors. In the maxillary arches this occurred during the eruption of the first incisors. An occasional "secondary" spacing of the upper deciduous anteriors took place when the still undeveloped maxillary arch was somewhat widened upon eruption of the permanent lower first incisors. Spaced deciduous arches generally produced favorable alignment of the permanent incisors while about 40 per cent of the arches without spaces produced crowded anteriors. Forward extension of the arches without spaces produced crowded anteriors. Forward extension of the arches was measured from a line connecting the distals of the canines. The averages revealed no difference between previously spaced and closed arches but were 1.0 mm. greater for the upper arches than for the lower ones. According to our tooth measurements this difference between the maxillary and mandibular forward extension could not be credited to a greater increase in thickness of the crowns of the upper permanent incisor over that of the lower ones. It was interpreted as an expression of an evolutionary tendency toward a lessened forward growth of the mandibular alveolar process.

87. IV. Ontogenesis of Incisal Overbite of the Mixed and Permanent Dentition. *Louis J. Baume, College of Dentistry, George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.* The development of overbite was surveyed in 52 cases each from 5 to 10 years of age. It was found that the degree of incisal overbite primarily was determined by the extent of mandibular forward growth which occurred during the eruption of the lower permanent incisors and later of the permanent canines and premolars. A lessened forward extension of the lower arch as compared with the upper one was responsible for a greater incidence of severe overbite in the mixed dentition. The deciduous overbite was also a determining factor in that the slight overbite tended to increase during the period of the mixed dentition while a severe overbite became worse. The definite overbite of the permanent dentition finally depended upon the eruption sequence of the permanent canines and premolars. If in the lower arch the sequence was canine, first premolar, second premolar and in the upper arch first premolar, canine, and second premolar, the best results were observed. Incisal overbite developed independently of the mechanism of molar adjustment. In conclusion the evidence of these 4 developmental studies shows that teeth and alveolar process are a genetic entity. Differentiation between general growth of the facial skeleton and that of the alveolar process shall be conducive for a better understanding of the etiology of malocclusion and the development of certain paradentopathies.

88. Old Age Changes in Human Parotid Glands With Special Reference to Peculiar Cells in Uncommon Salivary Gland Tumors. *William H. Bauer, School of Dentistry, St. Louis University, St. Louis, Mo.* Sixty-seven parotid glands removed at autopsy from individuals who died of various diseases and whose age ranged from 58 to 92 years were microscopically examined. Subserial sections disclosed, besides fatty metamorphosis and fibrosis, the occurrence and origin of the cells which constitute the so-called "onkocytoma" and the "clear-cell adenoma." The "onkocytes," more adequately named

"oxyphilic granular cell" (Meza-Chávez), are the product of a transformation of ductal epithelial cells into a special type with neoplastic potentialities. The change of acinous epithelial cells to "onkocytes" has not been observed. Ductal cells undergoing degeneration, thus revealing pyknotic nuclei in an oxyphilic cytoplasm, must not be confused with "onkocytes." The so-called "clear cells" result from a metamorphosis of the cytoplasm of ductal and acinous epithelium. The apparent "empty" cells are mostly hydropic or contain glycogen and are capable of neoplastic proliferation. Fundamental observations were also made concerning the development of the "papillary cystadenoma lymphomatosum."

89. Skeletal Changes in Severe Phosphorus Deficiency in the Rat. I. Tibia, Metacarpal, Costochondral Junction and Caudal Vertebra. *Russell D. Coleman, Hermann Becks, Freeke N. Kohl, and D. Harold Copp, College of Dentistry, George Williams Hooper Foundation for Medical Research, University of California, San Francisco and Berkeley, Calif.* Diets extremely deficient in phosphorus but containing adequate amounts of calcium and vitamins A and D were fed to 21 rats at time of weaning. Twenty were pair-fed a phosphorus supplemented diet and 8 given the control diet ad libitum. The tibia, third metacarpal, costochondral junction, ninth caudal vertebra, skull, teeth, and mandibular joint were studied roentgenographically and histologically. The very low phosphorus content of the diet was attained by employing iselectrically precipitated fibrin as protein. The calcium-phosphorus ratios of the deficient diets were 28:1, 31:1, 35:1, and 78:1. The latter ratio was decidedly lower than any previously reported. In spite of adequate amounts of calcium and vitamins A and D, severe rickets was produced in all bones. The pair-fed rats which were fed the diet supplemented with adequate phosphorus developed normal bones. The bones of rats fed ad libitum grew longer in length and showed more trabeculae formation. The severity of rickets increased as the animals advanced in age. However, a lowering of the phosphorus content from a calcium-phosphorus ratio of 28:1 to 78:1 did not seem to intensify the degree of rickets. The skeletal changes produced were the result of failure of the intercolumnar cartilage matrix and osteoid tissue to calcify. The failure of remodeling resorption of osteoid tissue increased the volume of osteoid and led to the characteristic picture of rickets. It must, therefore, be concluded that the absence of phosphorus was the most important factor in the production of rickets.

90. The Effect of Chronic X-ray Irradiation on the Rat Dentition. *Peter P. Dale, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Disturbed amelogenesis, dentinogenesis, delayed eruption, and periodontal pathology of the rat dentition was revealed with increasing, single, whole-body x-ray exposures (*J. D. Res.* **27**: 730, 1948). To ascertain the effects of chronic gamma radiation, 32 male and 32 female littermate albino rats, aged 75 days, were equally divided into 4 groupings and irradiated under constant conditions of 250 kilovolts, 8 or 15 milliamperes, 10 ft. 10 in. target-skin distance to: I-10 roentgen (r)/day, and II-20 (r)/day for 75 whole-body exposures, exclusive of Sundays, plus an acute dose of 600 r on the day following 75 treatments. Groups III and IV received 10 and 20 r daily, respectively, continuing beyond 75 exposures indefinitely. The oral tissues of moribund and surviving animals, sacrificed 60 days post exposure, were examined grossly (×30) and histologically by means of ground and decalcified sections prepared from respective halves of the jaws. Examination disclosed progressive disruption and retardation of enamel and dentin formation, retarded eruption rate, incisal attrition, periodontal and pulpal pathology with increasing exposures in all groups. Incisal pulps revealed early atrophy and degeneration

characterized by cystlike lesions and vacuoles, notchlike dentin, decrease in number and size of the odontoblasts, disrupted stellate reticulum, vascularity, and inflammation. Groups receiving the superimposed acute dose (I and II) had greater destruction. Ground sections revealed similar findings reported previously except for striated dentin characterized by alternate concentric striations of hypo- and hypercalcification. Molars showed large radicular pulps, smaller roots, decreased secondary dentinal, and cemental deposition. Typical circumferential erosion-like, gingival lesions, commonly seen in human teeth exposed to chronic irradiation, were absent.

91. Caries Susceptibility in Relation to the Fluoride Content in Drinking Water. *J. Danek and C. Parma, Governmental Dental Institute, Prague, Czechoslovakia.* The district of Kladno (Bohemia) has three geologic areas, viz., carbon, algonicium, and limestone with 1, 0.75 and 0.25 ppm F, respectively, in the drinking water. Six hundred eighty children of the largest schools in the carbon and limestone areas with 1.0 and 0.25 ppm F, respectively, in water were examined and caries index determined. In the area of less F the caries index was about 100 per cent greater than that in the area with ppm of fluorine. This demonstrates the importance of a sufficient amount of F in water for the prevention of dental caries.

92. Dental Caries in Orthodontic Anomalies. *J. Dudek, Prague, Czechoslovakia.* One thousand eight hundred and sixty school children between 6 and 12 years of age were examined for anomalies of the teeth and dental caries. It was found that Angle Class II had caries in 82 per cent of those examined, Class I in 39 per cent, and Class III in 57 per cent.

93. Oral Bacterial Flora in Miners. *J. Dudek, Prague, Czechoslovakia.* Of 450 miners, 9 per cent were infected by *Trichomonas tenax* and 4 per cent by *Endamoeba gingivalis.* Yeast and moulds were found in approximately one-half of the cases examined. *Penicillium assymetr* was especially frequent. There was a quantitative relation of the bacterial flora to the occurrence of both dental caries and periodontal diseases.

94. Aureomycin in the Therapy of Herpes Simplex Labialis and of Recurrent Oral Aphthae. *Frank G. Everett, University of Oregon Dental School and Medical School, Portland, Ore.* Prophylactic treatment with aureomycin ointment 5 per cent in a petrolatum base applied to the lips before and after dental treatment was administered to 289 patients receiving 902 dental treatments; a total of only 4 lesions of herpes labialis resulted, thus showing a reduction of more than 75 per cent in the postoperative incidence of herpes labialis when compared with a control group of 311 patients receiving 1264 dental treatments accompanied by lip protection with plain petrolatum ointment only, who developed 28 such lesions. In a second series 52 already existing lesions of herpes labialis in 44 patients were treated with repeated applications of aureomycin ointment 5 per cent. In all but 3 lesions discomfort was relieved immediately. A reduction (mean) of 39.9 per cent in the duration of the lesions was found when compared with the duration of the lesions previously experienced by the patients when indifferent or no therapy was used. In a third series 44 already existing lesions of recurrent oral aphthae in 38 patients were treated with up to 25 aureomycin troches (containing 15 mg. aureomycin each) administered orally every 2 waking hours over a period of 3 days. In all but 3 lesions discomfort and pain were relieved immediately. A reduction (mean) of 36.1 per cent in the duration of the lesions was found when compared with the duration of the lesions previously experienced by the patient when indifferent or no therapy was used. The best response to the aureomycin therapy in existing lesions of herpes labialis and of recurrent oral

aphthae was noted when medication started within the first 24 hours after eruption. (Published in *J. A. D. A.* **40**: 555, 1950.)

95. On the Alkaline Phosphatase Activity in the Enamel Organ of the Incisors of Vitamin E-Deficient Rats. *J. Glavind, E. Aaes-Jørgensen, H. Granados, and H. Dam. Polytechnic Institute, Copenhagen, Denmark.* Manganese, incorporated in the salt mixture, protects markedly against enamel depigmentation of vitamin E-deficient rats (Granados, Aaes-Jørgensen, and Dam; *Brit. J. Nutrition* **3**: 320, 1950, and *Acta path. et microbiol. Scandinav.*, in press). For testing the possibility that manganese might exhibit this protection through its influence on phosphatase, the alkaline phosphatase in the enamel organ of the depigmented incisors of vitamin E-deficient rats, and of controls, has been measured. Weanling rats reared for 10 weeks on vitamin E-deficient diets containing 20 per cent cod-liver oil and 10 per cent or 20 per cent protein, with their vitamin E-rich controls, were used. The middle, active portion of the enamel organ of both maxillary and mandibular incisors was dissected immediately after sacrificing the animals. The tissues, placed on glass slides, were dried in a vacuum desiccator over $CaCl_2$ for 24 hours. The samples were weighed, extracted in a tissue disintegrator with 4 c.c. aqueous phenyl phosphate solution, and incubated at 37° C. for 6 hours. Then 0.05 c.c. dichloro-quinone-chloroimide in alcohol were added, and after 15 minutes the extinction at 560 mμ was read in a Beckman spectrophotometer. The following average figures (extinction divided by weight) for the phosphatase activity of maxillary and mandibular enamel organ, respectively, were obtained. Group 1 (–E + 10 per cent protein), 62 (± 8.3) and 83 (± 17.6). Group 2 (+E + 10 per cent protein), 52 (± 2.7) and 58 (± 5.6). Group 3 (–E + 20 per cent protein), 61 (± 10.3) and 64 (± 2.6). Group 4 (+E + 20 per cent protein), 67 (± 4.0) and 97 (± 5.0). The results show that alkaline phosphatase activity in the enamel organ of the depigmented incisors of vitamin E-deficient rats is not significantly different from that of control rats fed vitamin E-rich diets.

96. Influence of Certain Substances on Incisor Depigmentation of Vitamin E-Deficient Rats. *Humberto Granados and Henrik Dam, Polytechnic Institute, Copenhagen, Denmark.* In vitamin E-deficient rats abnormal yellow-brown coloration of the adipose tissue and depigmentation of the incisors depend upon the feeding of certain highly unsaturated fatty acids (Dam and Granados: *Science* **102**: 327, 1945; Granados and Dam: *Proc. Soc. Exper. Biol. & Med.* **59**: 295, 1945). Comparing the mineral composition of depigmented incisors of rats fed a vitamin E-deficient diet high in cod-liver oil and low in protein with that of normally pigmented incisors of control animals, the following results were obtained: In depigmented incisors iron is very greatly diminished in both enamel and dentin, whereas manganese increases markedly in both tissues. Magnesium increases slightly in enamel but decreases in dentin. On the other hand, the percentage of calcium and phosphorus remains unchanged in both tissues (Dam, Granados and Maltesen: *Acta physiol. Scandinav.*, in press). Several experiments were carried out to study the influence of certain substances on incisor depigmentation and on peroxidation and coloration of adipose tissue in vitamin E-deficient rats. The following results on incisor depigmentation were obtained: (1) incisor depigmentation increases when low levels of protein are fed, and decreases gradually by giving progressively higher protein levels; (2) manganese, incorporated in the salt mixture, protects markedly against incisor depigmentation when combined with normal or high levels of dietary protein, but it does not offer such a protection when low protein levels are fed; (3) 0.5 per cent nordihydroguaiaretic acid, and 0.025 per cent antabus (tetraethylthiuramdisulfide) protect as 0.020 per cent tocopherol acetate against incisor depigmentation. The same protection is

offered by a vitamin E-deficient, rachitogenic diet high in calcium, but not by a similar diet high in phosphorus; and (4) incisor depigmentation reaches its maximum when the percentage of McCollum's Salt Mixture 185 is decreased from 5 per cent to 1 per cent.

97. Does Gonadectomy Influence the Caries Susceptibility on the Hamster? *Humberto Granados, Johs. Glavind and Henrik Dam, Department of Biology, Polytechnic Institute, Copenhagen, Denmark.* The influence of gonadectomy on caries activity of the hamster has been studied using 2 groups (10 males and 5 females in each) of weanling animals which were reared on a low caries-producing diet for a 100-day experimental period. All animals were weighed weekly. The caries activity of the 2 groups was as follows: Control males—average number of carious lesions 10.8, average caries score 6.8. Castrated males—average number of carious lesions 9.5, average caries score 6.8. Control females—average number of carious lesions 6.6, average caries score 3.5. Castrated females—average number of carious lesions 10, average caries score 7.1. It is concluded that under the established circumstances gonadectomy does not influence in any direction the caries susceptibility of the male hamster, but increases somewhat the caries activity in females. The growth curves of the 2 groups showed that gonadectomy increases markedly the growth rate and final body weight of both male and female hamsters. Since the present results are clearly opposed to what Keyes has found in his work on this subject *(New York J. Dent.* **18**: 172, 1948; *J. D. Res.* **28**: 653, 1949) it appears to be important that other investigators working under various experimental circumstances should carry out further studies on the possible effect of gonadectomy on caries activity. In this way it might be established whether under certain conditions different from those of the present experiment gonadectomy actually influences appreciably caries susceptibility.

98. The Effect of Water Soluble Chlorophyll on Mouth Organisms. *Barbara Griffiths and G. W. Rapp, Loyola University School of Dentistry Chicago, Ill.* Previous work has shown that water-soluble chlorophyll is able to lower the lactobacillus count of the mouth. Preliminary work has indicated that other microorganisms of the oral cavity are also adversely influenced by this medicament. This paper discusses the effects of water-soluble chlorophyll incorporated in a toothpaste and also in a mouthwash on the total oral flora as well as on individual types of oral bacteria. A mechanism is postulated to explain the observed results.

99. Geographic Variations of Dental Caries in Oregon. IV. Observations on First Molars as an Index of the Caries Experience in the Permanent Teeth of School Children 14, 15, and 16 Years of Age. *Demetrios M. Hadjimarkos and Clara A. Storvick, Oregon State College, Corvallis, Ore.* Data from dental examination records of a recent study (*J. D. Res.* **28**: 415, 1949) conducted among native-born and native-reared white school children, 14, 15, and 16 years of age, residing in Clatsop and Klamath counties of Oregon were analyzed for the purpose of determining the degree of accuracy by which their caries experience can be measured when 3 different technics are used. These are: (1) D.M.F. rates derived from observations on all permanent teeth, (2) first molar mortality rates, and (3) D.M.F. rates from observations confined to first permanent molars alone. Altogether 391 children, almost evenly divided by sex and age groups, were examined in both counties. The results obtained are as follows: D.M.F. rates: Clatsop 14.4, Klamath 9.0. First molar mortality rates: Clatsop 0.79, Klamath 0.81. D.M.F. rates of first permanent molars; Clatsop 3.91, Klamath 3.30. From the above it is concluded that within the age limits of the subjects considered in the present study, the caries experience of groups of school children can be determined accurately when ob-

servations are made on all permanent teeth. When consideration is given only to the mortality rates of first molars for measuring the degree of susceptibility to caries, the results can be misleading. Observations on the condition of the four first permanent molars alone result in an index of caries prevalence which may be regarded as of limited significance.

100. Rapid Analgesia in Dental Practice With Acetylsalicylic Acid-Benzosulfimide Combination (Theryl). *Murray M. Hoffman, Chicago, Ill.* The rapidity with which analgesia was obtained was studied in 584 patients, between 2 and 78 years of age, who complained of pain of dental or maxillofacial origin. Three hundred and fifteen patients were administered acetylsalicylic acid-benzosulfimide combination (Theryl); 269 patients (controls) were administered three other salicylate derivatives: acetylsalicylic acid, U.S.P.; acetylsalicylic acid, phenacetin and caffeine (A.P.C.), or acetylsalicylic acid, aluminum glycinate, and magnesium carbonate (Bufferin). Theryl was administered by permitting the tablets (10 grains) to disintegrate and to be absorbed sublingually without aid of water. The other 3 agents were swallowed by the patients with water. Analgesia was manifest from ½ to 5½ minutes with acetylsalicylic acid-benzosulfimide; 18 to 70 minutes was required for pain to disappear with the other 3 analgesics. Depth of analgesia was subjectively better with Theryl, probably because of greater rapidity of action. Duration of pain relief did not differ markedly for all agents used once analgesia was obtained. Sublingual administration and absorption of acetylsalicylic acid-benzosulfimide combination produces immediate and profound analgesia against pain of dental and/or maxillofacial origin.

101. A Progress Report on the Production of Microscopic Carieslike Lesions in Unerupted Hamster Molars in Vitro. V. *Hurst, J. Nuckolls, H. E. Frisbie, and M. S. Marshall, University of California College of Dentistry, San Francisco, Calif.* Experiments testing the ability of various bacteria to produce microscopic carieslike lesions in unerupted hamster molars in vitro are being continued. By January, 1950, histologic examination had been completed on 175 of the molars incubated in broth cultures for periods varying from 15 to 160 days. Twelve actinomycete strains, 4 lactobacillus strains, and 1 alpha streptococcus had produced lesions when the media remained at a neutral pH value throughout the test period. No lesions had been produced by *B. subtilis, B. aerogenes, B. coli, Sarcina lutea, Staph. albus, Staph. aureus, Z. zenkeri,* or oral diphtheroids under similar conditions. At the acid pH values obtained with lactobacilli and alpha streptococci in dextrose broth, the enamel was dissolved. Lesions were sometimes found among the remnants of enamel matrix. Additional tests employing 500 more hamster molars are in progress. These include 200 molars testing the effect of bacteria washed free of broth and suspended in physiologic salt solution. A few tests on autoclaved human enamel are also in progress.

102. A Preliminary Report on the Composition of the Organic Matrix of the Enamel. *W. E. Hutton and James Nuckolls, University of California, College of Dentistry, San Francisco, Calif.* Unerupted third molars were collected in distilled water within 8 hours of extraction. These were stored in the freezing compartment of an electric refrigerator. One-half of the crown of 1 of these molars was freed of dentin and surface material by attrition. The resultant enamel was washed 5 times in distilled water, dried in air, and weighed (0.15 gram). The enamel was then powdered and the powder extracted with 10 ml. of distilled water. The distilled water extract was then evaporated to 0.2 ml. Twenty μ spots (10-2 ul. applications) were made on sheets of Whatman No. 1 filter paper 11 × 11 cm. and chromatographed in 2 dimensions using water-saturated phenol as 1 solvent and as the other the epiphase of a

mixture of 40 per cent n-butanol, 10 per cent acetic acid, and 50 per cent water. A similar spot was tested with ninhydrin reagent (0.25 per cent triketohydrindene hydrate in water-saturated n-butanol) before chromatography. Although the unchromatographed spot showed a faint purple color, the chromatographed spots did not show any color, thus indicating the absence of free amino acids in this sample of enamel. (The faint color in the unchromatographed spot may have been due to NH_3.) The enamel, following the water extraction, was dissolved in cold 20 per cent HCl. The resultant solution was subjected to the same treatment as the water extract with the same results. This indicates that the enamel protein does not undergo immediate extensive hydrolysis at room temperature. After 2 hours' hydrolysis at 120° C. the following amino acids were tentatively identified by filter paper chromatography: cystine, glutamic acid, glycine, aspartic acid, serine, and tyrosine. Carefully controlled Mollisch tests indicated the presence in the organic matrix of the enamel of an unidentified carbohydrate, perhaps a glycoprotein, which was soluble in cold 20 per cent HCl and in dilute Na_2CO_3 (pH 10).

103. Caries in the Molars of the Rat Following Resection of Facial Nerve. Roentgenographic Study. *Joseph R. Jarabak, Northwestern University, Dental School, Chicago, Ill.* The purpose of this study was to determine the effect of food stagnation as a factor in dental caries. The facial nerve was resected at the stylomastoid foramen in 4 rats, 4 weeks old. This caused facial paralysis in all of the facial muscles. The buccinator muscle is primarily responsible for the transference of food from the buccal fold onto the tongue. With the severence of the nerve supply to this muscle, the food remains stagnated in this area. The rats were given a balanced fox chow diet. From the lateral roentgenogram the first evidence of caries can be seen between 7 and 9 weeks. The rarifaction begins in the distal surface of the third molar and continues mesially until the entire crown is destroyed, leaving the roots. In this group the destruction of the third molar varies from 21 to 26 weeks. At no time did we find any rarifaction in the second molars until the crown of the third molars were destroyed. In 7 rats of this group there was a rarifaction visible in the lateral roentgenogram in the second molars after the twenty-sixth week. The first molar has not shown any sign of rarifaction within the present limit of this experiment. Within the limits of this experiment we have not found an instance in which there has been any evidence of caries on the nonoperated side and in the control group. It appears as though the caries process is limited to the area of the third molar and the distal surface of the second molar due to lack of buccinator function. Continuation of this experiment for another 20 weeks may modify these findings.

104. Regeneration of the Mandibular Condyle in the Rat. *Joseph R. Jarabak, Northwestern University, Dental School, Chicago, Ill.* The purpose of this investigation was to determine the possibility of the regeneration of the mandibular condyle following resection. The mandibular condyle was resected unilaterally in 46 white rats and bilaterally in 33 rats at the age of 4 weeks. In each instance the resection was complete and included the neck of the condyle as well as the head. In 30 of the 46 unilateral resections there was either partial or complete regeneration depending on 2 factors, (1) the duration of time following the resection, and (2) maintenance of normal attritional pattern of the central incisors. In the 16 rats in which there was no regeneration, 5 showed complete or partial ankylosis from 9 to 19 weeks following resection, 7 expired within 1 month as a result of the surgical procedure; 4 animals died of starvation as a result of limited function due to continuous eruption of the central incisors. Of the 33 bilateral resections, 28 bilateral regeneration took place beginning 9 days after resection and carried out to completion from

8 to 17 weeks. The attritional pattern of the first incisors corresponds to that of the controls. In three rats of this group bilateral ankylosis occurred permitting the lower central incisors to erupt into the palate, limiting the function and causing starvation and death in the sixth and seventh week following resection. This experiment suggests that it is possible to have either partial or complete regeneration of the mandibular condyle in the white rat following a complete unilateral or bilateral resection.

105. Studies in Patients With Chronic Oral Mucous Membrane Lesions. *Maxwell Karshan, Daniel E. Ziskin, Herbert Silvers, George Stein, and Austin H. Kutscher. School of Dental and Oral Surgery, and College of Physicians and Surgeons, Columbia University, New York, N. Y.* Forty-three patients from 11 to 77 years with chronic soft tissue lesions in the mouth were investigated. Clinical examination of the tongue, cheek, hard and soft palate, floor of the mouth, and gingival tissues revealed either alone or in combination inflammation, desquamation, hyperkeratosis, blisters, ulcers, pseudomembranes, petechiae, edema, hypertrophy and atrophy, color abnormalities, excessive salivation, dryness, crusting, and bleeding. Considering these cases as a whole, increased blood values were found for (1) cholesterol in 36 per cent of the cases, (2) globulin in 28 per cent, (3) sugar in 20 per cent, and (4) erythrocyte sedimentation rate in 36 per cent, as compared with 9 per cent for cholesterol, 0 per cent for globulin, 2 per cent for sugar, and 19 per cent for erythrocyte sedimentation rate in a control group of 32. Decreased values for vitamin C were found in 28 per cent of the patients against 18 per cent in the control group. All blood values were essentially in the normal ranges for calcium, inorganic phosphate, phosphatase, total protein, albumin, vitamin A, carotene, nonprotein nitrogen, and hemoglobin. With the exception of the lymphocytes, which were somewhat about normal in 50 per cent of the cases, and the polymorphonuclears which were decreased in 30 per cent of the cases, the blood counts were in the main in the normal ranges. A similar result was obtained in the control group. The basal metabolic rate was outside the normal limits in 23 per cent of the cases. All patients gave a negative Kline test. On the basis of 1 week's food intake, it was calculated that the intake was low in the following percentage of cases for (1) calories in 68 per cent, (2) protein in 48 per cent, (3) calcium in 80 per cent, (4) phosphorus in 76 per cent, (5) iron in 48 per cent, (6) thiamin in 25 per cent, (7) riboflavin in 60 per cent, (8) niacin in 24 per cent, and (9) ascorbic acid in 20 per cent. Twenty-two control cases gave essentially the same dietary results. For 14 females between the ages of 14 to 60 years the average value for neutral 17-ketosteroids in urine for 24 hours was 6.9 mg. as compared with 10.7 mg. in a control group of 16 females between the ages of 20 to 54 years. Physical examinations revealed a large number of unrelated conditions; psychiatric interviews showed the presence of neurotic conflicts in a majority of the cases.

106. Protein in Basic and Acidic Human Saliva. *Frances Krasnow and Nina Budzinsky, New York, N. Y.* Analyses of total postabsorptive saliva were made on 240 cases ranging in age from 3 to 66 years. Their physiological conditions varied: 30 had no tooth involvement (I); 137 suffered from caries (C); and 73 indicated erosion lesions (E). Each of these groups was subdivided into those without systemic malfunction (designated ns) and those who complained of some generalized ailment (designated s) giving 18 Ins and 12 Is, 98 Cns and 39 Cs, 59 Ens and 14 Es. These 6 series were further classified with regard to salivary pH. The normal (Ins), implying no discernible tooth or other disturbances and 94.5 per cent manifesting pH 7 or above present a protein mean content equal to 281 mg. per 100 ml. Compared with this, elevated

concentrations were demonstrated by all other groups—the acid subdivisions being higher every time except for the one questionable caries-free, non-systemic individual. Obtaining pairs of figures will exemplify: Is-317, 334; Cns-330, 350; Cs-317, 400; Ens-327, 407; Es-386, 480. Moreover, the difference between the series for cases with systemic symptoms is greater than between those without any generalized illness as inspection of Cns and Cs or Ens and Es elicits. Values for D/S. E. mean, alkaline saliva caries or erosion groups minus mean, Ins equals 2.2 and 2.2, respectively, while D/S. E. mean, acid saliva caries or erosion series minus mean. Ins equals 4.7 and 5.0. Thus, aberrations in protein for salivas of pH 7 or above are probably real requiring additional case verification, whereas those for acid pH show absolute statistical significance.

107. Changes in the Dental Pulp of Children When Sodium Fluoride Paste Is Applied to Dentin Following Cavity Preparation. *Joseph P. Lazansky and M. Michael Cohen, Tufts College Dental School, Boston, Mass.* In previous investigations concerned with pulpal changes brought about by sodium fluoride, the chemical was applied to freshly cut dentin. In this study, observations were made on the young dental pulp when an interval was allowed to elapse between cavity preparation and the application of sodium fluoride. Thirty teeth were selected among children from 11 to 16 years of age who had to have first premolar teeth removed for orthodontic reasons. All teeth were free from caries and restorations. The first premolar in the maxilla or mandible was used as an experimental tooth, and the opposite tooth in the same jaw served as a control. Cervical cavities in 28 teeth were carefully prepared, making the size and depth as nearly alike as possible. Zinc oxide plus eugenol was inserted into the cavities of the teeth immediately following preparation. Thirty-three per cent sodium fluoride paste was applied for 10 minutes in 3 teeth, and sterile water was used to flush the cavity of the 3 control teeth, after which all cavities were sealed with zinc oxide plus eugenol. One to 20 weeks later, the zinc oxide plus eugenol was removed. Thirty-three per cent sodium fluoride paste was inserted into the cavities for 10 to 15 minutes in the 12 experimental teeth. In the 12 control teeth, the cavities were washed with warm tap water. Both experimental and control teeth were sealed with zinc oxide plus eugenol and were extracted at intervals of from 1 to 7 weeks. Very little difference was noted between sections from experimental and control teeth. The changes seen in the pulp varied from a slight reduction in the number of odontoblasts to vacualization of the odontoblastic layer and slight fibrosis. Some hemorrhage was seen in only 2 experimental teeth. In no instance was pulp necrosis or pulp abscess found.

108. A Comparison of the Size Discrimination of Disks by Teeth and Hands. *Lois F. Lunin and Barnet M. Levy, Washington University School of Dentistry, St. Louis, Mo.* The psychophysical nature of the oral cavity was studied by comparing the ability of the teeth and fingers to discriminate the size of small Lucite disks. Eighteen adult subjects were used. At specified time intervals, the test disks were placed either between the occlusal surfaces of the central incisors or the thumb and forefinger of either the right or left hand, and the subject was requested to state whether the disks were larger or smaller than a standard disk which had previously been used. If the subject failed to identify correctly the test disk, the same standard was reinserted followed by a test disk of different magnitude. This procedure was continued until the subject correctly identified the comparative size. Test disks measured 13 mm. in diameter and varied in thickness from 3 to 20 mm. in graded. intervals of 1 mm.; 4 standards arbitrarily chosen measured 5, 8, 12, and 17 mm. The results indicate that subjects can distinguish objects smaller than a

standard about 3 times more accurately with the teeth than with the fingers. Despite the many other factors to be considered, it may be possible to find some correlation between attrition of the teeth and failure of the subject to perceive or interpret the sensations received by the proprioceptors. The fatigue factor does not seem to enter into the discriminative ability of the subjects.

109. Relation of Gingivitis to Dental Caries and Malocclusion. *Maury Massler and Bhim Sen Savara, College of Dentistry, University of Illinois, Chicago, Ill.* The number of carious tooth surfaces, the number of malocclueded teeth, and the number of gingival units affected with gingivitis were analyzed in 2,774 children, 14 to 17 years of age, using the D.M.F. index of caries suggested by Klein and Palmer in 1938, the index of malocclusion as suggested by Massler (1950) and the P-M-A index of gingivitis as suggested by Schour and Massler in 1947 and 1950. Statistical analysis revealed that (1) no relation existed between the number of carious tooth surfaces and the number of inflamed gingival units and (2) no relation existed between the number of malocclueded teeth and the amount of gingivitis. However, children with severe gingivitis had a tendency to experience a higher caries attack rate as well as a larger number of malocclueded teeth than did the rest of the children.

110. Effect of Foods Grown in Different Areas on the Prevalence of Dental Caries in Hamsters. *Abraham E. Nizel and Robert S. Harris, Tufts Dental School, Boston, and Massachusetts Institute of Technology, Cambridge, Mass.* This study is an attempt to determine whether the development of caries in hamsters is affected by diets containing foods grown in different soil areas of this country. These areas have been shown to have a high and low incidence of caries from previous epidemiological surveys. Corn and milk were selected for study because these are commonly consumed and are suitable for use in a caries-producing diet. A modified Hoppert-Webber-Caniff diet of the following composition was used; corn 63 per cent, dried whole milk powder 30 per cent, alfalfa 6 per cent, and sodium chloride 1 per cent. The corn and milk components of Diet 1 were obtained from New England soils, and Diet 2 had corn and milk produced in southwestern Texas soils. The alfalfa, NaCl, and distilled water were constant factors in both diets. Two groups of Syrian hamsters, 13 males and 12 females in Group 1 were fed Diet 1 (New England) for 15 to 23 weeks after weaning and then sacrificed. A second group of 14 males and 11 females were fed Diet 2 (Texas) for 15 to 23 weeks after weaning and then sacrificed. The heads were fixed in formalin, dried, skinned, and treated with 3 per cent hydrogen peroxide. The teeth were examined, scored, and charted according to Keyes procedure. The scoring of caries was spot checked by other dentists with past experience in this technic. We observed that the male hamsters were more susceptible to caries than the females, and in all but 2 hamsters (both fed Texas diets) caries were found. The average number of carious teeth found in the New England group was 5.8 compared to 3.4 found in the Texas group—a difference of 2.4, or 41 per cent. The average number of carious surfaces in Group 1 (N.E.) was 7.4 compared to 3.9 for Group 2 (Texas)—a difference of 3.5, or 47 per cent. It is evident that the area in which foods are grown may affect the prevalence of dental caries.

111. Studies of Mastication. *C. Parma, University of Prague, Prague, Czechoslovakia.* A detailed study of human mastication was based on many anatomical investigations of the author as to the morphology of the masticating muscles, the skull, and the teeth in recent and prehistoric man and in animals. In addition, by functional examination of mastication, the author was able to confirm his anatomical results. The chief result of the study is

the demonstration of the existence of medial friction as the only rubbing movement of essential importance. The medial and lateral pterygoid muscles of the same side are explained as always working as a unit.

112. Penetrometers for Measuring the Radiopacities of Tissues. *Albert G. Richards, School of Dentistry, University of Michigan, Ann Arbor, Mich.* Two penetrometers are being used to measure the radiopacity or total density of the mandible and of lip tissue. Each instrument contains an alloy step-wedge of suitable thickness, an indicating caliper bearing a small ring at each contact, a bite-block, and means for holding a film packet behind the step-wedge and caliper rings. The osseous tissue penetrometer is positioned in the mouth with the caliper rings contacting the lingual and buccal surfaces of the mandible, thus measuring the mandible thickness. The instrument is retained by the patient when he bites on the bite-block portion of the device. The x-ray beam is directed so that the image of the measured thickness of the mandible will appear within the two concentric ring images. The step-wedge standard and the mandible of known thickness are radiographed simultaneously on the same film, thereby minimizing the effect of variations in processing and technic. The film density of the area of mandible appearing within the concentric rings and of the step-wedge is measured by an electronic densitometer. From the film density measurements, a certain thickness of the alloy standard can be determined which is the radiographic equivalent of the measured thickness of mandible. The radiopacity of this determined thickness of alloy is also the radiopacity of the measured thickness of mandible. The soft tissue penetrometer is used in a similar manner. A statistical analysis of the data obtained from measurements made on Caucasian patients with the osseous tissue penetrometer indicates that the radiopacity of the mandible, when measured between the premolar roots, increases with mandible thickness and is found to be higher than average for lean-faced individuals and below average for fat-faced persons. For very thin mandibles, this difference in the radiopacity of lean- and fat-faced individuals is negligible, but for thick mandibles the difference is very appreciable.

113. Effects of Temperature on Salivary Amylase Activity. *Leon H. Schneyer, Dental Department, Montefiore Hospital, New York, N. Y.* Freshly collected mixed human saliva diluted with 0.25 per cent NaCl comprised the enzyme solution. Somogyi's iodine method was used for the determination of amylase activity. The activity of salivary amylase increases rapidly as the temperature is raised to 50° C. Beyond 50° there is a rapid drop in activity. The increase in activity with a rise in temperature may be considered to result from the accelerating effect of temperature on the native amylase-starch reaction. The activation energy for this reaction is 17,654 calories. The appearance of the optimum and the decrease in activity as the temperature is raised are ascribed to the ascendency of a denaturation reaction. The activation energy of this process is 57,254 calories. The denaturation may be reversed by a reduction in temperature. There is an equilibrium between native and reversibly denatured salivary amylase, as there is with trypsin and bacterial luciferase. The denaturation process is slowed by the presence of substrate and by NaCl. The antidenaturant action of the substrate appears to be due to the combination with the native enzyme.

114. Tooth Extraction in Orthodontia. *F. Skaloud, University of Prague, Prague, Czechoslovakia.* In various countries, there are different views on extraction in the orthodontics. We shall only understand them if we study anthropology. The inhabitants of different parts of Europe, in spite of crossing of the races, preserve their distinguishing features in their physiognomy. They are, according to the cranial index, brachy-, meso-, or dolichocephalia. Accord-

ing to the facial index, they are chame-, lepto, or mesoprosopic. Besides, there may be orthognaty, mesognaty, or prognaty. In the north and south of Europe we find subdolichocephaly, in the neighboring countries mesocephaly and brachycephaly. In the east of Europe there is only brachycephaly. In dolichocephalics we find leptoprosopy, orthognatous face, protrusion of the nasal region, narrow and deep palate, small jaws, and teeth thrusting forward; the mandibular angle is obtuse. The teeth are too large to be held by a comparatively small apical basis and are crowded. This, we find in the Nordic race of tall stature and fair hair. Here, we can include the Iberoinsular race of short stature and brown hair. With brachycephaly, chameprosopy is associated. The face is orthognathous, thrusting forward in the nasal region, the palate broad and low, jaws of natural size, the alveolar process sits on a broad jaw. In the lower jaw, the mandibular angle is normal. There is enough room for the teeth in the apical basis. This we find in the Eastern, Alpinic, Celtoslavonic, and Mediterranean races. The Dinaric race of tall stature and brown hair is brachycephalic with meso- or chameprosopy. There is a tall, but short and broad head, orthognaty, the nasal region flattened or protruding. The jaws are mostly broad. The apical basis of natural size and the teeth are in regular position. Thus we understand why the nations of Nordic type have such a high percentage of orthodontic extractions. In regions with mesocephaly and mesoprosopy the dentists are cautious with regard to extractions. Most of Czechoslovakian children have a disposition to normal basis and the rate of orthodontic extractions is low, about 17 per cent.

115. Anomalies of the Teeth and Interference of Speech. *M. Sovak, Prague, Czechoslovakia.* Abnormal configuration of the hard palate (high or gothic), 6.6 per cent, was found in 3,350 children of preschool age and 7.2 per cent in 1,343 children of early school age. These abnormalities were associated with hyporhinolalia (difficult nasal breathing) in 12 per cent of the first group and 92.8 per cent of the second group. Adenoids were found in 85.7 per cent and 82.2 per cent, respectively. Anomalies of occlusion were found in 4.92 per cent of the first group and in 6.4 per cent in the second group. The various forms of malocclusion were associated with hyporhinolalia in a very high percentage of cases (71.4 to 100 per cent) and adenoids were the causing factor in 62 to 100 per cent. Thus anomalies of the hard palate and malocclusion leading to the narrowing of the nasal and nasopharyngeal cavities show a tendency to interfere with the nasal breathing and predispose to the growth of adenoids.

116. The Effects of a Protein-free Diet on the Teeth and Periodontium of the Albino Rat. *George Stein and Daniel E. Ziskin, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* (Abstract published with Scientific Proceedings of New York Section of International Association for Dental Research, May 5, 1949: *J. D. Res.* **28**: 529, 1949.)

117. A New Instrument and Method for Reducing Toothbrush Abrasion. *Alvin E. Strock, Boston, Mass.* The need for an effective, nontraumatic instrument for toothbrushing, which requires a minimum of cooperation, dexterity, time, and need for instruction, is recognized. This paper deals with the development of such an instrument for tooth brushing which can nontraumatically be used for effective cleaning of the teeth, following the natural horizontal direction of motion for brushing. This concept is based upon the reduction of friction by the bristles and dental tissues, although fully adequate for prophylaxis and ideal for a nontraumatic gingival stimulation, if indicated. A cervical damage is reduced and embrasures are more readily reached, the need of patient education is diminished, and manual dexterity is not a requirement. The use of this principle makes it possible to effectively use such fine strands

of bristle, or nylon, that the advantages of each can be embodied in construction. Photomicrographs show that damage by abrasion is due to trauma from the irregularity within the tuft primarily, in addition to the shape of the individual bristle end.

118. Decoloration of Blue Starch Solution as a Test for Caries Susceptibility. *J. Svejda, and C. Budejovice, Czechoslovakia.* Velocity of decoloration of 0.1 per cent blue starch solution (colored blue by iodine) was studied by the modified method of Wohlgemuth for diastase determination in urine. Decoloration of the solution mixed with the saliva of 16 caries-resistant persons was quicker than when mixed with the saliva of 27 caries-susceptible ones.

119. The Influence of Fluoride on the Fracture Healing Process in Young Rats. *Joseph C. Tam, School of Dentistry, University of Minnesota, Minneapolis, Minn.* The influence of 20 and 100 parts per million of sodium fluoride given ad libitum in distilled water on the fracture-healing process of the fibulae was studied histologically by decalcified celloidin sections stained with haematoxylin and eosin and was tested by the modified breaking strength device of Lindsay. Forty rats were distributed into 2 divisions, histological and healing strength. Each division was subdivided into 5 equal groups. The controls were given distilled water. Some of the experimental rats were given sodium fluoride only after the fracture and some were given sodium fluoride before and after the fracture. The synthetic diet fed ad libitum contained 0.63 ppm of fluoride. After 77 days on this regime, the left fibulae were fractured during ether anesthesia with a modified Hertz's plier. The fracture sites were verified with roentgenographs. In some cases, a second fracture attempt was necessary resulting in multiple simple fracture of the fibula. One rat in each group from each division was sacrificed at intervals of 10, 17, 24, and 31 days after the fracture. The sections in the 5 groups from the histological division were comparable and showed no significant cellular and structural differences in the fracture healing process. In the 5 groups from the healing strength division, the frequency of break occurred randomly at and away from the original fracture site, such that this plus other uncontrollable factors made a conclusion impossible.

120. Reinclusion of a Permanent Molar. *K. Wachsman, Professor of Dentistry, Governmental Dental Institute, Prague, Czechoslovakia.* A rare case of a secondary retention of the upper first permanent molar in a girl of 14 years is described. It was caused by the eruption of the upper third molar and proceeded so quickly that in the course of 3 months only the occlusal third of the tooth was to be seen. Three months after the upper third molar had been extracted, the submerged tooth spontaneously reached the level of occlusion again.

121. Rapid Diffuse Penetration of Intact Enamel and Dentin by Carbon 14-Labeled Urea. *W. W. Wainwright and F. A. Lemoine, Los Alamos Scientific Laboratory of the University of California and the Los Alamos Hospital, Los Alamos, New Mexico.* Radioautographic studies of 14 freshly extracted human teeth which were painted with radioactive urea and sectioned under oil revealed rapid diffuse penetration through intact and carious enamel and dentin. It is assumed that it is the urea molecule which has penetrated the teeth but since the radioautograph detects only the radioactive carbon, further experiments will be necessary to substantiate this assumption. The property of diffuse penetration is doubtless related to the high diffusibility of urea and its small molecular size which confirms the prediction of earlier workers that enamel is more permeable to small molecules. Four teeth showed penetration into the pulp. Penetration through enamel into the dentin was more

frequent near the gingival line and near occlusal fissures. Radioactive urea and the radioautographic technic are highly effective tools for use in extending studies of enamel permeability. The combination provides a means of grossly visualizing the pathways by which nonstaining substances of small molecular size penetrate enamel. Since urea penetrates enamel rapidly and deeply it would seem that it can precede the bacteria of decay in their progress into the tooth. It is possible, therefore, that urea can exert its bacteriostatic and enzyme-inhibiting effects in and beyond any invaded portion of a tooth and thereby impress a severe handicap on the growth of the invading organisms and the damaging effects of their enzyme products. This hypothesis can be extended to postulate a class of substances of small molecular size which enter enamel freely and deeply. Some of these may have the power to inhibit, while others may enhance caries activity. (Published in *J. A. D. A.* **41**: 135, 1950.)

122. Relation Between X-ray Tube Potential and Diagnostic Value of Dental X-ray Film. *Arthur H. Wuehrmann and Richard S. Manly, Tufts College Dental School, Boston, Mass.* Previous investigations concerning technics of dental radiography have usually employed x-rays generated at tube potentials ranging from 35 to 70 kilovoltages peak (kvp). The purpose of this study was to compare the diagnostic value of dental films at tube potentials ranging from 30 to 110 kvp. The criterion of an ideal radiograph is considered to be one which demonstrates soft tissue and at the same time produces good differentiation of calcified structures of nearly equal radiographic density. Highly calcified structures must, at the same time, have greater film density than metallic restorations. The study made use of an aluminum step-tablet (with 19 1-millimeter steps), no-screen x-ray film, and a Weston densitometer. By trial and error, the correct exposure in milliampere seconds was determined for each 5 kvp from 30 to 110 kvp as required to keep the density constant for film under 2 millimeters of aluminum. Eight by 10 no-screen film was exposed under the complete step-tablet at the chosen mas. for each 5 kvp from 30 to 110. The density of film under all steps was determined from the average of 5 readings. At 110 kvp adjacent steps under all parts of the tablet could be noted visually; at 30 kvp the gradations under the thicker parts of the tablet were too slight to be discerned. These procedures were repeated: (1) using the living skull in a lateral position with the step-tablet placed in front of the nose in the mid-sagittal plane, and (2) using the same film intraorally with the step-tablet place equally distant from the tube target. Evidence has been obtained that the radiopacity range in the mouth corresponds to the first 14 steps of the step-tablet. If this is true, in order to demonstrate soft tissue and at the same time produce good differentiation between structures of greater densities, it is necessary to utilize tube potentials in excess of those commonly found in dental x-ray equipment.

123. The Range of Histological Variation of Clinically Normal Gingivae. *Leo Zach, Montefiore Hospital, New York, N. Y.* Twenty gingival biopsies clinically described as normal were subjected to histological examination with the purpose of describing the range of structural variations none of which might be ascribed to periodontal disease. Marked differences in both the epithelial and connective tissue elements of the gingivae were found in the various specimens, as well as variation in gross thickness of biopsies from similar sites. Epithelial variations described include thickness of the entire layer, relative sizes of the component layers, degree and type or absence of keratinization, parakerotosis, uniformity of surface. Cytological differences cover vacuolization in the prickle cell layer, presence or absence of a granular and lucid layer, clarity of tonofibrils, and appearance of the germinative layer. The connective tissue is considered with regard to size and distribution of

papillae, presence of a clearly defined lamina propria, type of fibrillar structure, cellularity, vascularity, and extent of round-cell infiltration. Deviation in one or more histological aspects from the classical descriptions of gingiva in most of the specimens studied was the rule. The variations described cannot be correlated with pathologic conditions and are to be classified as normal variants of healthy gingiva.

XII. Executive Proceedings and Register of Newly Elected Officers and Members

A. COUNCIL SESSIONS

Activities Between General Meetings

Between the Twenty-seventh and Twenty-eighth General Meetings, the Council determined the time and place of the Twenty-eighth General Meeting, nominated the Council candidates for general officers for inclusion on the official ballot form, approved the detailed Secretary-treasurer's budget, and received various reports and memoranda without comment.

First Session, Friday, 4:30 P.M., March 24, 1950

Council meeting called to order by President Blayney with an attendance of 20. Received and approved the reports of the Secretary-treasurer and the financial reports of the Publication Committee (p. 702). On motion these reports were approved. Received the report of the treasurer of the Committee on the William J. Gies Endowment Fund for the *Journal of Dental Research* for the period between June 24, 1949, and March 17, 1950 (p. 704). On motion this report was approved; in addition, it was ordered that this report be read at a Saturday session, printed in the proceedings, and that Dr. Waugh as Treasurer and Dr. Arthur H. Merritt as Chairman of the Committee be cordially thanked for their efforts in the interest of this endowment and in the welfare of the *Journal of Dental Research.*

After a long discussion concerning annual programs, place and time of the general meeting and the finances of the Association, on motion the President was directed to appoint a committee of three to consider program policy, membership of the program committee, the publication of abstracts both before and after the general meeting and to report back to the Council at the Sunday morning session. On motion the formation of new sections in the Kansas City area and in Denmark was approved. Adjourned until Sunday morning.

Second Session, Sunday, 8:30 A.M., March 26, 1950

Meeting called to order by President Blayney with an attendance of 23. The report of the committee on program was called for (Bibby, Chairman, Dean and Kitchin). They recommended that the by-law dealing with the program committee should be changed so that the principal responsibility for arranging the meeting should rest with President of the Association, who should be the chairman of the program committee, that the program committee should have responsibility for the selection of papers for inclusion on the program of the annual meeting, and that as circumstances dictate the program committee could, without the necessity of consulting the Council, have the privilege of modifying the program of the annual meeting. In the ensuing discussion it was disclosed that all recommendations of the committee except the one dealing with the chairmanship of the program committee were consistent with current practice and supported by pertinent sections of the by-laws. It was then moved and seconded that a proposal be made at the closing executive session to amend the by-laws to the effect that the President shall be the chairman of the program

committee instead of the Secretary-treasurer. The discussion disclosed no serious objection to such a change and that the proposal had the support of the Secretary-treasurer. The motion was carried by a vote of 12 to 8, with the direction that the chairman of the committee draft the proposed amendment to the by-laws and propose it at the closing executive session.

It was moved and seconded that the present practice of preparing and reproducing before the meeting short abstracts of all program items listed for oral presentation be continued. Carried.

Chairman Hill of the committee appointed to validate and count the votes on the proposed amendment to the Constitution reported that the amendment had been adopted by a vote of 337 to 64. (Any vote over 312 is valid.) The copy of the amendment follows:

Article VIII. Finances. Section 1. *Amount of dues.*

At each annual meeting of the ASSOCIATION the amount of the annual dues, payable by each member before the close of the succeeding annual meeting, shall be determined and announced by the Council, and may not exceed ten dollars ($10.00).

The report of Chairman Hodge of the Committee on the membership register was received and the committee continued until the register is published.

The report of the Board of Editors including an account of the proceedings of their recent meeting, Saturday, March 25, was received and on motion was ordered approved and filed for printing in these proceedings. It was moved and seconded that the Council indicate its complete approval of the contract with The C. V. Mosby Company for another three-year period and specifically of the proposed increase in the number of pages to be printed annually of 600 to 900 and a corresponding increase in the subscription price of $4.00 to $6.00 per year.

By formal motion duly seconded and carried, the Council authorized the formation of a standing committee to consider nominations to membership to the ASSOCIATION and to submit periodic reports on nominations to the Council. Furthermore the Council directed that although the committee appointments are to be made annually, members are eligible for repeated terms.

The report of the committee appointed to study the nominations for membership reported a list of persons fully eligible for membership. In the ensuing discussion no additional names were added to this list and on motion it was decided by a unanimous vote to certify these persons to the ASSOCIATION for formal election to membership. (See list printed on p. 707.)

It was moved and seconded that the Council favor a time in the last two weeks of March as the time and Chicago as the place for the 1951 General Meeting. Carried by a divided vote.

By motion duly seconded the following budget for the 1950-1951 year was unanimously approved:

Dues assessment for North American members	$7.50
Dues assessment for members residing outside of North America, if and when possible	$1.00
Remission of dues of charter, 20-year, and emeritus members.	

Disbursements:

For support of the activities of the office of the Secretary-treasurer, up to	$700.00
For support of the activities of the Publication Committee, up to	$1,800.00
For membership subscriptions to the *Journal of Dental Research* at the rate of $6.00 per member, up to	$3,300.00

There being no other business before the Council and supported by a motion duly passed, President Blayney adjourned the Council sessions for the 1949-1950 year.

The following Councillors attended one or both sessions of the Council held on March 24 and 26: Agnew, Arnim, Arnold, Bevelander, Bibby, Boyle, Blayney, Burrill, Bryan, Costich, Crawford, Dobbs, Fosdick, Hall, Hatton, Hill, Hine, Hodge, Jay, Kitchen, Noyes, Peyton, H. Robinson, Swanson, Zander. Total, 25.

B. REPORT OF THE PUBLICATION COMMITTEE AND THE BOARD OF EDITORS OF THE *Journal of Dental Research*, 1949 (VOLUME 28)

The Editor reported that Volume 28 of the *Journal of Dental Research* contained 697 pages with 76 original papers, 139 abstracts, 6 Editor's Viewpoints, the Executive Proceedings, the index, and miscellaneous matter. The additional space was made available by the purchase of 97 pages above the contract agreement (600 per volume) from money received as a royalty from the printer. Despite the large numbers of papers published in 1948 and 1949 (161), publication time from receipt of manuscripts is almost one year.

The Board of Editors met at French Lick Springs Hotel with the following present: Dunning (Boston), Kitchin (Columbus), Scott (Washington), Jay (Michigan), Becks (San Francisco), Lovestedt (Minnesota), Easton (Iowa), Clough (Richmond), Taylor (Materials Group), Hatton (I.A.D.R.), Arnim (Publication Committee), Robinson, presiding, and Hall, Secretary.

The problem of requests for large numbers of reprints was discussed and the Editor was instructed to use his discretion when such requests came from large research institutions which customarily order more than 500 reprints not for commercial advertising purposes. The change in contract between The C. V. Mosby Company and the Board of Editors increasing the volume size to 900 pages was read and the mail vote of 21 for approval (a few with minor changes) and 1 against was reported. The vote carried. The percentage of papers rejected and reasons for rejection were discussed. After discussion, it was agreed that the "Editor's Viewpoint" should be continued.

Dr. Hatton reported that a vote taken to amend the Constitution to permit maximum dues of $10.00 per annum had been favorable and that there are now 640 members in the ASSOCIATION. Possible means of increasing the number of subscribers to broaden the influence of the *Journal* was discussed. It was suggested that the publisher be approached to consider preparation of subscription books to be used by members to obtain additional subscriptions. [A report received subsequently showed that there were 2,421 subscribers in April, 1950, vs. 2,262 in 1949. There are approximately 2,100 copies circulated in the United States and 500 in foreign countries.]

C. EXECUTIVE SESSIONS

First Session, Saturday, 9:15 A.M., MARCH 25, 1950

The session was called to order by President Blayney with a brief welcome to members and guests. The minutes of the Twenty-seventh General Meeting were offered as printed in the *Journal of Dental Research* in the December, 1949, number, pp. 627-688. Since no corrections were offered to the printed record, the minutes of the 1949 meeting were declared approved as printed in the *Journal of Dental Research*. The official election for general officers was opened for the reception of ballots and a committee of three tellers appointed

to receive the official votes and count them. (Arnold, Burrill, Knighton.) The resignations of Arno B. Luckhardt and W. H. Sebrell were announced and also the loss of five members by death (Moses Diamond, Bernhard Gottlieb, Percy R. Howe, Arthur P. Little, and T. W. Osborne).

Under the heading of financial reports, statements were read from the Publication Committee, from the Treasurer of the Committee on the William J. Gies Endowment Fund for the *Journal of Dental Research*, and from the Secretary-treasurer. The first two are printed elsewhere in the proceedings (pp. 702 and 704, respectively), the last follows:

Receipts for the 1949 Calendar Year

From dues of members	$2,193.80	
From the C. V. Mosby Company	1,800.00	
From interest	17.50	
From Chicago Section collections	84.00	
Total		$4,095.30

Disbursements

For expenses of the Secretary-treasurer	$ 553.95	
For expenses of the Publication Committee	300.00	
For member subscriptions to *J. D. Res.*	1,984.00	
For refunds for duplicate collections	18.80	
For extra pages in *J. D. Res.*, paid to The C. V. Mosby Company	1,500.00	
Total		$4,356.75
1949 Deficit		$ 261.45

Funds on Hand as of December 31, 1949

General fund, checking account, State Bank and Trust Co., Evanston, Ill.	$1,571.94	
General fund, savings account	1,022.20	
Total		$2,594.14
I. A. D. R. endowment fund, established by W. J. Gies, savings account #8538	$ 238.20	
Bonds	1,300.00	
Total		$1,538.56
One U. S. savings bond	25.00	
Grand total on hand December 31, 1949		$4,157.70

The above reports were approved for inclusion in the minutes of this meeting. By a unanimous vote Drs. Arthur H. Merritt and Leuman M. Waugh, Chairman and Treasurer of the William J. Gies Endowment Fund for the *Journal of Dental Research* were cordially commended for their interest in and services for this fund. The executive session was then adjourned until the Sunday evening session.

Second Session, Sunday, 8:15 P.M., March 26, 1950

The ASSOCIATION was called to order by President Blayney and the report of Council activities made the first order of business. These were reported by the Secretary-treasurer as printed elsewhere in the proceedings (p. 700) and included therein are the following specific items: (1) Report of the committee appointed to count and verify the vote on the proposed constitutional amendment, carried by a vote of 338 in favor of the amendment to 64 against it. (See *J. D. Res.* **27**: 777, 1948, for text of amendment.) It raises the limit on dues from $5.00 to $10.00 per year. (2) Approval of the budgets and proposed dis-

bursements for 1950-1951 as printed in the council proceedings. (3) A dues assessment for North American members of $7.50 and of $1.00 for members outside of North America for the 1951 calendar year; the 1950 dues assessment for new members elected at this meeting was set at $6.00 per member including a 1950 subscription to the *Journal of Dental Research*.

Disbursements for the 1950-1951 year up to Dec. 31, 1951, were authorized as follows: (1) Secretary-treasurer's office for the period Jan. 1, 1951, to Dec. 31, 1951, $700.00. (2) Expenses of the Publication Committee, Jan. 1 to Dec. 31, 1951, including additional pages in the *Journal of Dental Research* over the contract allowance of 900. (3) Member subscriptions to the *Journal of Dental Research* at a block rate of $6.00 per North American members for the 1951 volume, with an additional $0.50 to each foreign subscription.

A list of candidates for membership as submitted by the Council as having all needed qualifications for membership was read as printed elsewhere in these proceedings (page 707). On motion and by unanimous consent the provisions for a formal written ballot were waived and the Secretary-treasurer instructed to cast a vote for the persons whose names are carried on this list.

A written proposal was offered by Dr. Bibby requesting formally that Part B of Article 2 of Section C of the by-laws be amended. The current draft follows: "The Secretary-treasurer shall be chairman of the Program Committee." The proposed amended form provides that: "The President shall be chairman of the Program Committee." In the ensuing discussion Dr. Bibby urged its adoption on the basis that this proposed arrangement would favor greater and very desirable variations in the annual programs. The proposal was also supported by the Secretary-treasurer. After reading Section 2 of Article XII of the constitution the proposed amendment was carried without a dissenting vote.

On motion the State Bank and Trust Co. of Evanston, Ill., was directed to honor checks and withdrawals from ASSOCIATION funds held in checking and savings accounts belonging to the ASSOCIATION only on the order and signatures of any two of the following ASSOCIATION officers during the period between March 29, 1950, and March 15, 1951: President Basil G. Bibby, Secretary-treasurer Edward H. Hatton, and President-elect Leonard S. Fosdick. The Secretary-treasurer was then directed by unanimous motion to draft and transmit letters of appreciation to those individuals and groups who had done so much to facilitate the conduct of this meeting, specifically listing: the management of the French Lick Hotel, and especially Mrs. Gladys Howell and Mr. Davis; Dean Hine of the University of Indiana and the very skilfull operator for the unusually efficient projection service they provided; the ladies who served voluntarily at the registration desk during the periods of active registration. There being no other business the chairman proceeded to the closing exercises.

These began by reading the list of the losses in membership caused by death since the date of the last General Meeting, a group distinguished by long service and outstanding contributions in the area of dental research. At his request the audience stood briefly in grateful tribute to the memory of: Moses Diamond, Bernhard Gottlieb, Arthur P. Little, T. W. Osborne, and Daniel E. Ziskin.

The newly elected officers, listed elsewhere in these proceedings (p. 707), were then called to the speaker's platform and formally inducted into office by the outgoing President, Dr. Blayney, who then called for the inaugural address of the new President, Dr. Basil G. Bibby, of the Eastman Dental Dispensary, Rochester, N. Y., at the conclusion of which Dr. Bibby received the gavel, called for new motions, and there being none, formally adjourned the 1950 General Meeting.

D. WILLIAM J. GIES ENDOWMENT FUND, JOURNAL OF DENTAL RESEARCH

Financial Statement as of March 17, 1950

Compiled by LEUMAN M. WAUGH, D.D.S., Treasurer

This Fund was created, on the initiative of the voluntary Committee on Endowment—organized in New York City in 1937—to insure the continuance of the *Journal of Dental Research* "in full accord with the highest ideals of strictly professional journalism devoted to the advancement of research," to which the *Journal* was dedicated at its establishment in 1919.

I. *Amounts Received From Organizations*
(June 24, 1949, to March 17, 1950)

Ohio State Dental Society	$60.00	
		$60.00

II. *Amounts Received From Individuals*
(June 24, 1949, to March 17, 1950)

None	
Total contributions 1949-1950	$60.00

III. *Interest, Income and Expense (current year)*

No expenses were recorded.

INTEREST TO DATE, MARCH 17, 1950, ON WAR BONDS:

Number of Bonds	Denomination of Bonds	Date Purchased	Interest Accrued During Current Year	
10	$1,000.00	March 1942	$260.00	
22	1,000.00	May 1942	286.00	
8	1,000.00	July 1942	208.00	
2	1,000.00	Dec. 1942	26.00	
1	1,000.00	March 1943	26.00	
1	25.00	Feb. 1944	.63	
5	1,000.00	March 1944	125.00	
2	1,000.00	May 1944	24.00	
1	1,000.00	July 1944	23.00	
3	1,000.00	Sept. 1945	57.00	
2	1,000.00	March 1946	32.00	
Total				$ 1,067.63
Total contributions 1949-1950 (Section I and Section II)				60.00
Total addition to the Fund, including accrued interest for the current year				$ 1,127.63

IV. *Summary of the Total Assets: 1937-1950*

Total receipts (assets) for the Fund as of March 17, 1950		$47,579.35
Paid for 56 War Bonds (Section VI)	$41,440.00	
Shares of stock (Section VI)	365.00	
Interest accrued during current year	1,067.63	
Interest accrued during 1946-1949	3,765.20	
Balance		$ 941.52

V. *Cumulative Summary of Annually Recorded Items of Receipts and Expenditures*

Year	Contributions (Payments)	Earned Interest	Expense	Net Receipts	Cumulative Annual Net Receipts
1937-38	$10,652.50	None	$134.06	$10,518.44	$10,518.44
1938-39	4,748.17	None	None	4,748.17	15,266.61
1939-40	5,693.78	None	None	5,693.78	20,960.39
1940-41	5,190.55	$ 206.78	3.53	5,393.80	26,354.19
1941-42	4,166.35	333.95	None	4,500.30	30,854.49
1942-43	1,766.31	132.19	None	1,898.50	32,752.99
1943-44	3,302.23	314.00	None	3,616.23	36,369.22
1944-45	3,744.45	459.05	None	4,203.50	40,572.72
1945-46	1,144.50	497.17	None	1,641.67	42,214.39
1946-47	249.50	1,203.28	None	1,452.78	43,667.17
1947-48	77.63	1,217.40	None	1,295.03	44,962.20
1948-49	145.00	1,344.52	None	1,489.32	46,451.72
1949-50	60.00	1,067.63	None	1,127.63	47,579.35
	$40,940.97	$6,775.97	$137.59	$47,579.35	

VI. *Statement Regarding Investment in War Bonds*

By authorization of the Trustees of the ASSOCIATION, fifty-six War Bonds of Series F ("12-year appreciation bonds"), costing $740 each, have been purchased from the funds in the Endowment Committee's Treasury, as of the following dates and serial numbers:

Purchased (10) March	1942:	M140400F to M140409F, incl.	$ 7,400.00
Purchased (22) May	1942:	M197811F to M197832F, incl.	16,280.00
Purchased (8) July	1942:	M237558F to M237565F, incl.	5,920.00
Purchased (2) December	1942:	M423990F and M423991F	1,480.00
Purchased (1) March	1943:	M204356F	740.00
Purchased (5) March	1944:	M750832F to M750836F, incl.	3,700.00
Purchased (2) May	1944:	M752375F and M752376F	1,480.00
Purchased (1) July	1944:	M892919F	740.00
Purchased (3) September	1945:	M649868F to M649870F, incl.	2,220.00
Purchased (2) March	1946:	M1061926F and M1061927F	1,480.00
			$41,440.00

The payee named on each bond is "Committee on the William J. Gies Endowment Fund for the *Journal of Dental Research* (an unincorporated body)." All of these War Bonds are in the custody of the Treasurer of the Committee, in a safety deposit box, Empire Trust Company, 580 Fifth Avenue, New York 19, N. Y., in trust for the Committee.

The contribution of $18.50, listed in Section II of the report for 1943-1944 from Dr. Joseph E. Psayala, was in the form of a $25 United States War Bond Series F ("12-year appreciation bond"), Q631670F, purchased in February, 1944, the payee being "William John Gies Endowment Fund for the *Journal of Dental Research.*" It is in the custody of the Treasurer in a safety deposit box, Empire Trust Company, 580 Fifth Avenue, New York 19, N. Y., in trust for the Committee.

The Farnsworth Television and Radio Corporation Stock, indicated in Section IV, is also in the custody of the Treasurer of the Committee, in a safety deposit box, Empire Trust Company, 580 Fifth Avenue, New York 19, N. Y., in trust for the Committee.

VII. *Summary of Open Accounts Relating to Pledges*

	Pledge	Paid	Balance	
A. Organizations				
Kansas: First District Dental Society	$ 100.00	$ 25.00	$ 75.00	
J. B. Mann Study Club, Washington, D. C.	1,000.00	500.00	500.00*	
				$575.00
B. Individuals				
Harry Kaplan	250.00	100.00	150.00	
R. J. Rinehart	200.00	75.00	125.00	
				$275.00
				$850.00

Respectfully submitted,
LEUMAN M. WAUGH, Treasurer

E. OFFICERS AND MEMBERS ELECTED

ACTIVE OFFICERS: President—*Basil G. Bibby*, Eastman Dental Infirmary. President-elect—*Leonard S. Fosdick*, Northwestern University. Vice-president—*Maynard K. Hine*, University of Indiana. Secretary-treasurer, *Edward H. Hatton*, Northwestern University. Trustee (for a five-year term)—*Paul C. Kitchin*, Ohio State University. Editor—*Hamilton B. G. Robinson*, Ohio State University. Additional members of the Publication Committee—*J. Frank Hall*, University of Indiana, and *Sumter S. Arnim*, University of Texas.

MEMBERS ELECTED: Bethesda, Md.—*Robert C. Likens*, *Morrison Rogosa*, and *Albert L. Russell*, National Institute of Dental Research. Boston, Mass.—*Leonard Robinson*, Tufts College. Chicago, Ill.—*S. N. Bhaskar*, *M. S. Burstone*, and *Frank M. Wentz*, University of Illinois. Copenhagen, Denmark—*Jens J. Pindborg*, National Danish College of Denmark. Glen Ellyn, Ill.—*Iden N. Hill*, Zoller Memorial Dental Clinic. Indianapolis, Ind.—*Thomas M. Boyd* and *Marjorie L. Swartz*, University of Indiana. Johannesburg, South Africa—*Alfred John Clement*, University of Witwatersrand. Los Angeles, Calif.—*Bernard Z. Rabinowitch*, University of Southern California. Minneapolis, Minn.—*William D. McBride*, University of Minnesota. Philadelphia, Pa.—*J. T. Rothner*, Temple University. Pittsburgh, Pa.—*Ida M. Harman*, Vernon-Benschoff Co. Richmond, Va.—*J. C. Forbes*. Rochester, N. Y.—*John W. Hein*, and *William G. Shafer*, University of Rochester. Seattle, Wash.—*Alton W. Moore*, University of Washington. Washington, D. C.—*Walter C. Hess*, Georgetown Medical and Dental School and *Aubrey C. Swaney*, National Bureau of Standards. Waterloo, Iowa—*Gilbert F. Glasson*, recently with the National Bureau of Standards. Total, 23.

F. MEMBERS VOTING AT THE ANNUAL ELECTION

Agnew, Aisenberg, Allen, Anderson (B. G.), Anderson (G. M.), Appleton, Archer, Arnim, Bagnall, Bauer, Bavetta, Bernick, Bernier, Beube, Bibby, Bier, Blackerby, Blackwell,

*In previous reports the J. B. Mann Study Club of Washington, D. C. has been credited with having paid $200 of its pledge of $1,000.

About ten days ago, it was called to our attention that the Mann Club had cancelled checks showing that a total of $500 had been paid. Each of these checks was endorsed with the rubber stamp of the Endowment Fund which proves that they were deposited to the Fund's account. Since no checks have been drawn *by* the Committee on the William J. Gies Endowment Fund (the *Chairman and Treasurer* must sign such checks), all of the payments of the Mann Club have been included in the total receipts as reported previously.

Some of these checks had been sent to the office of the Chairman who deposited them direct. It is our opinion that, in interoffice communication and due to change of office personnel, two of these checks were credited to the officer signing the check instead of to the Mann Club as an organization. We now are in correspondence to learn the names of the persons who signed the checks. The totals of the report will remain unchanged.

Blayney, Boyd (D. M.), Boyle, Bradel, Brand, Brandhorst, Brashear, Brauer, Breitner, Broadbent, Brodie, Brown (J. B.), Brown (J. C.), Brucker, Bunting, Burkett, Burrill, Carr, Caul, Chase, Cheyne, Chilton, Clark, Clawson, Clough, Cohen (J. T.), Cohen (M. M.), Coolidge (T. B.), Costich, Cox, Crawford, Cross, Dahlberg, Dale, Darlington, Dean (H. T.), Deatherage, Dietz, Dobbs, Dragiff, Dreizen, Dressel, Dummett, Dunning (J. M.), Easlik, Easton, Edwards (L. F.), Edwards (R. W.), Elliott (F. C.), Elliott (M.D.), Ellis (R. G.), English, Enright, Epstein (I. A.), Erikson, Everett, Fisher, Fleming, Fosdick, Freeman, Frey, Gabel, Gafafer, Gilda, Ginn, Glickman, Goldberg (H. J. V.), Goldman, Goodsell, Grossman, Gruebell, Gurley, Hadjimarkos, Hahn, Hansen, Hill, Hirschfeld, Hodge, Hoffman, Hooper, Howell, Hubbell, James, Jarabak, Jasper, Jay, Jordan (L. G.), Jordan (W. A.), Kaiser, Kaletsky, Kaplan, Keilty, Kerr, Kesel, Keyes, Keys (D. A.), Khalifah, Kitchin, Kniesner, Knighton, Koch, Koser, Koski, Krashen, Kreshover, Krogh, Kurth, Lalonde, Land, Lasater, Lefkowitz, Leonard, Levy, Lieber, Losch, Lovestedt, Lowery (P. C.), Lowry (R. A.), Ludwick, Lund, Lundquist, Lura, Lynch, Maier, Main, Mandel, Manly, Mann, Margolis, Martin, Mason, Massler, McBride, McCall, McCauley, McCollum, McCrea, McKay, Merkeley, Mercado, Midgley, Milhon, Millberry, Miller (S. D.), Miner, Mitchell, Moore (Mary), Moore (N. A.), Moore (T. E.), Morse (Anna), Morse (F. W., Jr.), Mueller (A. H.), Mueller (Emil), Myers, Nelson, Neuwirth, Nizel, Noyes (F. B., Noyes (H. J.), Nuckolls, Nylander, Oartel, Edith Oblatt, Oliver (C. P.), Orban, Orland, Ostrom, Owen, Patterson, Pearlman, Dorothy Permar, Peterson (H. W.), Peterson (L. W.), Peyton, Phatak, Phillips, Podolin, Prinz, Rabkin, Dorothy Radusch, Rapp, Rault, Ray, Robinson (H. B. G.), Rogers, Rutherford, Ruzicka, Ryder, Salzman, Sarnat, Schantz, Scherer, Schlack, Schoonover, Seltzer, Shaw, Shell, Shourie, Sicher, Hannah Silberstein, Simmonds, Simmons, Simon, Skinner, Sloman, Smith (E. L.), Smith (R. V.), Smyd, Snyder, Sorrin, Speidel, Stafne, Steadman, Stein, Stewart, Swanson (H. A.), Swanson (W. F.), Sweet, Taber, Taylor (N. C.), Taylor (W. T.), Telford, Thoma, Thomas (B. O. A.), Thompson (G. Earl), Thompson (J. R.), Thomson (H. S.), Tiblier, Van Huysen, Volker, Volland, Wach, Waggener, Wainwright, Wallace, Walter, Waldron, Ward, Waugh, Weinmann, Wessels, Wheeler, Betty White, Wilbur, Willett, Willman, Williams, Wolfe, Worman, Wylie (W. L., Sr.), Zander. Total, 268.

G. MEMBERS IN ATTENDANCE AT ONE OR MORE SESSIONS, LISTED BY SECTIONS.

Ann Arbor—Jay, Peyton. *Baltimore*—Dobbs, McCrea. *Boston*—Barr, Dunning, Hatch, Glass, Glickman, Manly, Marshall-Day, Sognnaes, Waldo, Zander. *Chicago*—Blayney, Brodie, T. Coolidge, Engel, Fosdick, Freeman, Harrison, Hatton, Kesel, Koser, Krashen, Ludwick, Martin, Orland, Rapp, Romnes, Rovelstad, Schour, Sicher, Skinner, Teuscher, Tilden, Tylman, Wach, Wallace, Weinmann. *Cleveland*—Bruckner, Chase, Hill, Kniesner, Pearlman, White. *Columbus*—Ennever, Kitchin, Permar, Robinson. *Houston*—Arnim. *Indianapolis*—Hall, Hine, Muhler, Phillips, Van Huysen. *Iowa City*—Boyd, Bryan, Easton, Smith, *Kansas City*—Myers, Scrivener. *Los Angeles*—Rutherford. *Louisville*—Burrill. *Minnesota*—Clark, Crawford, Lovestedt, Mitchell, Simon, Speidel. *New York*—Bevelander, Karshan, Levy, Miller, Stein, Taylor, Wright. *Oregon-Washington*—Noyes, Thomas. *Philadelphia*—Appleton, Boyle, Cobe, Gabel, Taylor, Williams. *Pittsburgh*—Cox, Strader, Swanson. *Richmond*—Clough, Kreshover. *Rochester*—Bibby, Brudevold, Costich, Hodge. *San Francisco*—Agnew, Becks, Nuckolls, Sloman. *St. Louis*—Boling, Brady, Brandhorst, Gurley, Knighton. *Tennessee*—Ginn. *Toronto*—R. G. Ellis. *Washington*—Arnold, Bernier, Caul, Dean, English, Hampp, Losee, McClure, Oartel, Paffenbarger, Pfeiffer, Rault, Schlack, Schoonover, Scott, Souder, Stephan, Sweeney, Zipkin. *Not members of a section*—Brauer, Brumfield, Holmes, T. E. Moore, Nelson, Pedersen, Volker, Wessinger. Total, 132.

Attendance of New Members, Elected March 26, 1950—T. M. Boyd, Burstone, Forbes, Harman, Hein, Hill, Likens, McBride, A. W. Moore, Rabinowitch, Rogosa, Shafer, Swaney. Total, 13.

Total registered attendance, including guests, 286.